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COMPOSITION OF WHEAT IN RELATION TO STAGE OF MATURITY AND EXPOSURE TO FROST

Arthur Gilbert McCalla
Department of Field Crops

University of Alberta
April, 1931

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The undersigned hereby certify that they have read and recommend to the Committee on Graduate Studies for acceptance a thesis on "Composition of wheat in relation to stage of maturity and exposure to frost," submitted by Arthur Gilbert McCalla, B.Sc., in partial fulfilment of the requirements for the degree of Master of Science.

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COMPOSITION OF WHEAT IN RELATION TO STAGE OF MATURITY AND EXPOSURE TO FROST

Arthur Gilbert McCalla
Department of Field Crops

A THESIS

submitted to the University of Alberta
in partial fulfilment of the requirements for
the degree of

MASTER OF SCIENCE

Edmonton, Alberta
April, 1931

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COMPOSITION OF WHEAT IN RELATION TO STAGE OF MATURITY AND EXPOSURE TO FROST

Arthur Gilbert McCalla

INTRODUCTION

Every year a certain proportion of Western Canada's wheat crop is frozen before reaching maturity, and the effect of such freezing on the chemical composition and milling and baking quality of the grain is of great practical importance in relation to the problem of equitable grading and merchandizing. In the fall of 1928, an unusually large percentage of the crop was damaged by frost. The grades were low, the yields reduced in some cases, and the financial loss to the farmers exceedingly heavy. The Associate Committee on Grain Research of the National Research Council undertook a study of the nature of this damage to wheat and to its value in flour and bread making. The first year's work was confined to the analysis and study of commercial samples collected from the 1928 crop. The results of this investigation are now being prepared for publication.

In 1929, a freezing plant was installed at the University of Alberta and artificially frozen samples of

known history obtained. Unfortunately, these gave very little definite information as the effect of the frost was much less severe than had been intended. The experiment was repeated with much greater success in 1930. Chemical studies and milling and baking tests are being carried out on the samples so obtained, the milling and baking tests being in part cooperatively handled by the Universities of Alberta, Manitoba and Saskatchewan. These results are too voluminous to be included in this paper and will be referred to only in passing.

Since it was intended to compare the frozen samples with unfrozen checks, it was considered desirable to obtain as much fundamental information as possible concerning the normal course of ripening in wheat. It was decided, therefore, to study a shorter series than that used in the frost work from the standpoint of stage of maturity only.

Time did not permit a comprehensive study of the chemical development of wheat with reference to all constituents, and this paper is chiefly concerned with changes in the content of dry matter and various nitrogen fractions. The results of the analyses of samples in the maturity and in the artificially frozen series are presented and discussed in detail in this paper.

LITERATURE REVIEW

General

The study of the chemical development of wheat and the effect of frost on this development has received most attention during the last twenty years or so, but there are numerous earlier publications dating back as far as 1864. The earliest papers to which reference has been found were published by French workers at about this time. Most of the earlier literature dealing with frozen wheat and appearing from 1885 to 1900 is concerned with the feeding value of the frozen grain, but the analyses are worthy of note.

There are numerous questions concerning this subject which have not yet been satisfactorily settled or adequately investigated. As in all such studies there have been, and still are, many complicating factors. A clear interpretation of analytical results is often difficult. Many phases of the development of wheat that have received the attention of other workers have not been touched on in this investigation. Only those papers dealing directly with this study will be reviewed.

Grade, Germination, Weight per Bushel

According to Shutt (34), the grade of wheat cut

on successive dates gradually improved as maturity approached until the field was frozen. The wheat from the last cutting before the frost graded No. 2 Northern, but that from all later cuttings which should have been more mature and presumably as high or higher in grade, fell to Nos. 4, 5, 6 and feed. Frost and not immaturity was therefore responsible for the lowering of the grade. These findings are supported by the work of Johnson and Whitcomb (20) which showed that the grades of frozen samples were lower than for the corresponding checks where such were available.

It has been found that very immature kernels are capable of almost perfect germination, but that frost cuts down the percentage of viable seeds, (20, 38). The reduction in germinating ability depended on the severity of the frost and the stage of development of the wheat when frozen. As a general rule it was found that the more mature the grain at the time of freezing, the higher the percentage of germination.

Arny and Sun (2) found that the weight per bushel increased gradually as development progressed, but that from the time the wheat reached the hard dough stage until it was ripe the change was insufficient to affect the grade. The weight per bushel of frozen wheat has been found by Johnson and Whitcomb (20) and Sharp (29) to be lower than that for check material, but Whitcomb and Sharp (39) report the opposite relation in immature samples.

Fresh and Dry Weight

The percentage moisture content of wheat kernels has been found to decrease more or less regularly from the time of flowering until maturity, but the actual weight of water present in developing grain at first increases, soon reaches a roughly constant level, and then drops rapidly, (12, 27, 41). Brenchley and Hall (12) found that this constant weight period was about 18 days long, while Woodman and Engledow (41) reported it as 10 days. These latter workers further determined that the fresh weight of the kernels increased until rapid desiccation set in. At the same time a distinct change in kernel colour from green to yellowish-brown could be noted. The weight of dry matter increased until the same time, but from then on changed little until the final week, during which period a measurable decrease was recorded. The fact that the changes in weight of dry matter are slight after the dry matter reaches 56-60% of the kernel weight has been substantiated by the work of Arny and Sun (2), Olsen (24), Saunders (25, 26) and Sharp (27). Arny and Sun, however, report that although changes are slight after this stage has been reached, there is a gradual rise until maturity, but that translocation of materials from the vegetative parts to the kernel ceases as soon as the grain

is cut. This latter contention is supported by the work of Wilson and Raleigh (40) with oats. The results obtained by Shutt (33) on the other hand, indicate that since the weight per 1000 kernels of wheat dried in the stook was higher than that of wheat threshed before drying, there must have been translocation of food materials after cutting.

Booth (9) found that the dry weight of oats increased markedly until the fifteenth day of his experiment, but that the subsequent increase was only 3%, while the loss sustained by delaying harvesting two days after the ~~wheat~~ oats would normally have been cut was 8.3%. Harlan (19) found that raw materials of barley were translocated until a late stage of development. His "ripe" sample, however, contained only 58% dry matter, and hence his results are of no value in determining whether or not translocation occurs after the grain reaches the stage of rapid desiccation.

That there is a critical stage in the development of wheat seems fairly well established. Any changes which occur after this stage is reached are apparently due to desiccation rather than to other factors.

Nitrogen Fractions

Total nitrogen. Early workers have reported that

the percentage total nitrogen in wheat kernels decreased as the grain matured, and explained this decrease by the theory that nitrogenous materials are deposited earlier in the development of the kernels than carbohydrates, and hence as the carbohydrate substances are moved into the grain the nitrogen percentage of necessity decreases. One of the references frequently quoted as substantiating the existence of this relation is that of Deherain and Meyer (14). These workers, however, did not analyse the grain alone, and the relation that they found between nitrogen and carbohydrate elaboration applies not to the grain, but to the whole plant. Their results with heads of wheat indicate that there is a rise in percentage total nitrogen toward maturity. The erroneous citation of their work may have arisen from a misreading of their paper by an early reviewer, whose mistake has been perpetuated by later copyists.

There are other papers, however, in which the same idea has been advanced. The experimental results obtained by Kedzie (21), Shutt (31, 32), and more recently by Arny (1), Arny and Sun (2), and Olson (24) indicate that there is a fall in percentage total nitrogen during the development period.

Most of the modern work is in disagreement with these results, revealing instead that although there is a

decrease in percentage total nitrogen from the early stages of development, this decrease does not long persist, and that a distinct rise occurs toward the end of the development period. The amounts of the early decrease and later increase have been found to vary a good deal in separate experiments, but the fairly consistent occurrence of this relation seems to justify the belief in its existence.

Brenchley and Hall (12) reported this course for total nitrogen, but chose to explain it in the following manner. The ratio of nitrogenous to non-nitrogenous material moved into the endosperm is constant during the whole development period; the bran or pericarp layers, which are formed first, are higher in nitrogen than the endosperm, and as the latter is formed within the pericarp the percentage nitrogen of the kernel decreases; the final increase in nitrogen percentage may be explained by the loss of carbohydrates in respiration. Thatcher (35, 36) more or less agrees with this explanation. In his results, the final percentage nitrogen content was higher than the original, but there was a decrease during the early stages. Woodman and Engledow (41) present similar figures for kernels analysed in the fresh condition, and Saunders (25, 26) found the decrease and subsequent increase in dried material, though in one of his series the ripe sample

did not attain the value of the original. Miss Brenchley (11) reported a very similar course for total nitrogen in barley.

Brenchley (10) also made a cytological study of the developing wheat kernel and concluded that the reserve nitrogenous material was moved into and laid down in the kernel at the same time as the starch, and that there were no apparent differences during the course of development.

The actual weight of nitrogen and protein in the developing kernels has been studied by Saunders (25) and Thatcher (35, 36). The former found that the protein content per 1000 kernels rose from 0.884 to 5.104 gm. during development, that the rise was most rapid during the early stages, and that protein and carbohydrate materials seemed to be deposited at relatively the same rate. Thatcher found similar changes, but the amount of protein was very nearly constant during the latter days of the ripening period. Brenchley (11) reported similar relations in the development of barley proteins.

The effect of respiration on the total nitrogen content of wheat has been studied by McGinnus and Taylor (22) and Tottingham (37). The former workers measured the loss of carbon dioxide by respiration, and calculated the actual loss of carbohydrates. They concluded that as much

as 10 gm. of material could be given off during a normal 16-day ripening period. They used threshed kernels in their determinations so the results are probably not entirely comparable to those which would be obtained under field conditions, but they do prove that the loss of carbohydrates is relatively great. These workers further calculated that such a loss would cause an increase of 1.46% in the protein content of the wheat. This increase due to respiration is great enough to explain the actual rise found in protein content of wheat under normal conditions. Tottingham found that respiration losses were largely influenced by temperature. Since the ratio of respiration to synthesis was greater at higher temperatures than at lower, the grain grown at the higher temperatures would contain a higher percentage total nitrogen or protein than that grown under cooler conditions. What is most important, however, he found that the increased nitrogen percentage was apparently due to the loss of carbohydrates by respiration of the kernels.

Early workers reported that frozen wheat was higher in total nitrogen than non-frozen (18, 20, 31, 32), and attributed this to the immaturity of the wheat when frozen. Others have found the reverse situation with the greatest differences in the most immature kernels (28). Johnson and Whitcomb conclude from their own work and from a survey of the literature that the differences in

percentage nitrogen content of frozen and non-frozen wheat are due to factors other than frost, and that the percentage may be higher or lower in the frozen wheat depending on these factors.

These same workers found that the total nitrogen content of flour milled from frozen wheat bore a normal relation to that of the wheat, but Sharp (28) found that some of the flours from frozen samples were higher in total nitrogen than the original wheat. All of his checks, on the other hand, were quite normal. In all wheat which contained 54% or more dry matter at the time of freezing, however, he found that the nitrogen content in frozen wheat and the flour milled from it agreed fairly well.

Salt-soluble nitrogen. Woodman and Engledow (41) studied the nitrogen of wheat soluble in 1% NaCl, and found that the percentage decreased from the first formation of the kernels until the wheat had reached the stage at which it contained about 55% dry matter, and from this point on remained fairly constant. Sharp (27) found that frozen wheat, especially those samples which were immature when frozen, contained higher percentages of nitrogen soluble in 5% K_2SO_4 than did the unfrozen checks.

Non-protein nitrogen. The non-protein nitrogen in wheat follows a very similar course to that of the salt-soluble nitrogen except that the amounts are smaller,

according to Woodman and Engledow (41). There was very little change during the last few days, and the fact that any of these lower compounds remain as such is ascribed to trapping of them in the rapidly developing tissues where they have no chance of taking part in synthetic processes.

These lower nitrogen compounds are present in larger amounts in frozen than in non-frozen wheat (5, 20, 32), the largest differences being found in the most immature grain. Johnson and Whitcomb suggest that when frost stops synthesis the non-utilized compounds persist in the dried kernels, while in non-frozen material at a similar stage of development synthesis proceeds to a much greater extent during the drying process.

Shutt (32) reported that when frozen wheat was milled, these differences in non-protein nitrogen were not observed in the flour, but Blish (5) and Sharp (28) state that most other work is in disagreement with this view. Shutt milled only those wheats which had been but lightly frozen, and which, while they showed external frost damage, may not have been frozen severely enough to affect the endosperm.

Gluten

Woodman and Engledow (41) report that the most immature fresh kernels showing distinct gluten formation contained 55% dry matter at the time of cutting. The amount of gluten increased as the grain became riper.

Crude gluten determinations have been made on frozen and check samples by Johnson and Whitcomb (20) and Whitcomb and Sharp (39). They found that in the early samples the dry gluten percentage was higher in the checks than in the frozen material, that in the more mature samples the values were about equal, and that the gluten content in all wheat which had reached a dry matter content of 54-56% before it was frozen was unchanged by the frost.

Whitcomb and Sharp found that in every sample analysed the percentage dry gluten was less than the percentage crude protein calculated as total nitrogen x 5.7. The results of Dill and Alsberg (15) and Olson (23), however, indicate that in some cases the percentage dry gluten is higher than that of crude protein.

Ash

The findings of Johnson and Whitcomb (20), Kedzie (21) and Woodman and Engledow (41) indicate that

the percentage ash content of wheat decreases as the grain ripens. The results obtained by Johnson and Whitcomb for frozen wheat indicate that the ash determination is not significant except as a measure of maturity.

In the foregoing review no reference has been made to glutenin or gliadin fractions although these have been discussed in many of the papers cited. As a result of work done at this University, some of which is reported in the paper, it was decided to drop the routine glutenin determination, and this decision has recently been strengthened by the recommendation of Blish (7) to the Association of Official Agricultural Chemists that this determination be discontinued until there is obtained a more definite knowledge of what glutenin really is.

An attempt to harmonize some of the conflicting ideas found in the course of this review will be made in discussing the experimental results of the present study.

ANALYTICAL METHODS

Dry Matter

In fresh kernels and flour. The dry matter content of fresh kernels and flour samples was determined by drying the material in a vacuum oven at 98° C. for 48 hours.

In dried wheat. The dry matter in all dried wheat was determined by drying a 2-gm. sample in an air oven at 130° for 1 hour after the material had been ground in a Wiley mill.

Total Nitrogen

All total nitrogen determinations were made by the Kjeldahl method using mercuric oxide as the oxidizing agent and a 2-hour digestion period.

Ammonia Nitrogen

A 20-gm. sample of fresh kernels was ground as finely as possible with sand and 20-50 cc. distilled water in a mortar, then transferred to a distilling flask with enough distilled water to make the volume up to 250 cc. The suspension was made slightly alkaline with $Ca(OH)_2$ and warmed to 50° C. Acid-washed air was drawn through the suspension for 1 hour, the ammonia being collected in N/70 sulphuric acid after passing through a condenser and Folin-bulb. A few drops of caprylic alcohol were added every 15 minutes to prevent foaming in the flask.

This method is based in general on the procedures used by Van Slyke and Thimman for the determination of ammonia from hydrolysates.

Salt-soluble Nitrogen

In fresh kernels. A 10-gm. sample of fresh kernels was ground with sand and approximately 20 cc. of 5% K_2SO_4 in a mortar, then transferred to a 250-cc. centrifuge bottle with sufficient 5% K_2SO_4 to make the total volume of liquid up to 200 cc. The bottle was shaken mechanically for half an hour, then the contents were centrifuged. The resulting supernatant liquid was clear and filtering unnecessary. A 50-cc. aliquot was pipetted into a Kjeldahl flask and used for nitrogen determination.

In dried wheat and flour. The above procedure was modified for dried, ground wheat and flour, by using 8 gm. of dry matter and shaking with 200 cc. of 5% K_2SO_4 for 1 hour.

Non-protein Nitrogen

A 100-cc. aliquot of the supernatant liquid from the above salt extraction was pipetted, with continual shaking, into a flask containing 100 cc. of 2.5% trichloracetic acid. The flask was allowed to stand for half an hour, then the contents were filtered. A 100-cc. portion of the clear filtrate was used for a Kjeldahl nitrogen determination.

This general method has been used by Bishop (3) with barley extracts, but he found that 2.27% trichloracetic acid was the best concentration to use, and states that varying this concentration affected the amount of nitrogen precipitated. No such variations could be noted with wheat extracts, using concentrations of the precipitant from 2 to 5%. This agrees with the results of Greenwald (17) who used this reagent as a precipitant for blood proteins. He found that a concentration of nine times the normal strength of his precipitant gave no difference in results. Bishop centrifuged after precipitation, but it was found here that a water-clear filtrate could be obtained without centrifuging, and that absorption of higher compounds by kaolin was so slight that this step as suggested by Bishop was not used in routine analysis.

Water-soluble Nitrogen

A 5-gm. sample of dry matter was shaken for 1 hour with 125 cc. of toluene-saturated distilled water. The suspension was centrifuged, and 100 cc. of the supernatant liquid used for Kjeldahl nitrogen determination.

Washed Gluten

Dill and Alsberg's method (15) for washed gluten was modified by using tap water instead of the sodium phosphate solution. Although the pH of this water is approximately 7.5 and the salt concentration is 0.02%, a comparison of the modification with the original method showed no appreciable difference in results obtained for dry gluten, although those for wet gluten were slightly lower as determined by the modification.

Ash

The ground samples were ashed in a muffle furnace at 570° C. for 6 hours.

EXPERIMENTAL MATERIAL

Series 1: Maturity Series, 1930

Production and collection. This series consists of 19 samples of wheat which were collected from a small section of a 5-acre plot of Marquis wheat grown on clean fallow land at Ellerslie (near Edmonton) in 1930. On July 21, the day on which the heads on most of the central

culms were in full bloom, 5000 heads in as nearly as possible the same condition were marked with small tags. This provided a base from which to accurately determine the age of all grain subsequently collected from the plot. The collecting started on July 26 and continued for two weeks after the wheat would normally have been cut on Sept. 2. Collections were made at 8.30 in the morning, the heads being cut from the culms, put into a covered can and taken to the laboratory. Approximately 300 heads were collected at the earlier cuttings, this number gradually being decreased to 150 as the weight of the grain increased. One-third of the heads was dried at once in an air oven with a rapid circulation of air at 60° C. The kernels were immediately removed from the remainder, half of them being used for analyses in the fresh state, and the other half spread out on plates and allowed to dry at room temperature. These air-dried kernels and the kernels threshed from the heads dried at 60° C. were ground successively in a Wiley and an Arcade mill. The resulting fine powder was later used for analyses.

Notes on the samples. Samples M 1 to M 4 were collected while the kernels were still forming and hence small, thin and watery. The dry matter content was constant during this kernel formation.

The kernels in samples M 5 to M 7 made a steady, rapid increase in plumpness, but the contents remained watery, and the original green color of the kernels persisted.

The contents of the kernels in samples M 8 to M 11 gradually changed from watery to milky, and from milky to a fairly firm dough. The color gradually changed from green through yellowish to a decided pink. When the kernels dried slowly they acquired a clear amber color with a slight greenish cast.

Sample M 12, cut on Aug, 22, was made up of completely pink kernels which dried to a clear amber, and which were plump and firm. This sample was the first to show a tenacious gluten pad formation when the kernels were ground with water in a mortar.

The kernels in samples M 13 to M 17 exhibited a gradual change in texture from firm to very hard, and in color from pink to a bright clear amber. All the kernels when dried were clear amber in color.

The kernels in samples M 18 and M 19 were very hard, but had lost the clear brightness of the amber color which characterized M 16 and M 17.

Further details as to dates of cutting, dry matter content and results of the various analyses will be taken up in a later section.

Series 2: Frozen Series, 1929

Production and collection. Four varieties of wheat, Garnet, Reward, Marquis and Renfrew were grown on fallow land at West Salisbury (near Edmonton) in 1929,

and collections were made at various stages of maturity of the wheat, artificially frozen samples being produced from each. Cutting was started at the time the wheat contained about 40% dry matter and was continued until it was ripe. The plots were long and narrow, and each cutting consisted of one swath the entire length of the plot.

About 45 sheaves of each of two varieties were cut as early in the morning as conditions would allow. These were tagged in rotation as they fell from the binder: F.A., F.B., and Check, and immediately hauled to the freezing plant which consisted of two chambers, and therefore made it possible to freeze two lots at different temperatures each day. Each chamber contained two racks of chicken-wire shelves 6 inches apart with a capacity of 10 sheaves of wheat cut open and spread out not more than 2 inches deep. The F.A. and F.B. sheaves were spread out on the shelves in the inner and outer chambers respectively, and the ice machine started. The air in each chamber was circulated by means of a Sirocco fan connected with flues opening at various levels. An attempt was made to have the resulting temperature curves approximate those of outdoor temperatures on a night on which frost occurred. The samples were cooled to temperatures ranging from 30° F. to 18° F., the minimum temperatures being maintained for one to two hours, after which the machine was shut off and the doors opened.

The actual freezing schedule for the Garnet and Reward samples was as follows:

Sample		1	2	3	4	5	6	7	8
Dry matter at cutting		41.5	42.9	49.0	48.2	52.6	54.3	56.6	63.0
Freezing temp. °F.	F.B.	28	29	26	29	30	26	24	23
	F.A.	25	27	24	27	28	24	22	18

That for the Marquis and Renfrew samples was very similar.

Shortly after the doors of the freezing chambers had been opened, the sheaves were retied and, with the unfrozen check samples, stooked outside under natural conditions, except that weathering changes were precluded as much as possible by covering the stooks with canvas covers whenever there was rain, and at night. As soon as the grain was sufficiently dry, it was threshed. Each sample was carefully cleaned, special precaution being taken to avoid the loss of small, injured kernels. These samples were then used for analytical, milling and baking studies.

Notes. The year was very dry and the yields of Garnet and Reward were light, but there was sufficient material of every variety to complete the series. Eight cuttings each of Garnet and Reward, and nine of Marquis and Renfrew were made. This gave a series containing 102 samples. The results from this series were very disappointing as the frost treatment was too light to cause appreciable damage.

Very few of the results will be included in this paper, as their value does not begin to equal their volume.

Series 3: Frozen Series, 1930

Production and collection. Only one variety, Marquis, was used in this series, which is made up of 79 samples collected from a 5-acre plot of wheat grown on clean fall land at Ellerslie (near Edmonton). This plot has already been mentioned in connection with Series 1.

The technique used in collecting these samples was very similar to that used in 1929, but the freezing technique was modified. The chambers were cooled to 28° and 24° F., and 22° and 18° F., respectively on alternate days, and the minimum temperature was maintained for 4 hours. The machine was shut off at the end of the minimum temperature period, but the rooms were allowed to remain closed and the wheat not removed until the following morning. The temperature therefore rose gradually, more nearly approaching natural conditions. There were occasional minor variations from the temperatures mentioned, and such variations are noted in the tables in a later section of the paper.

The sheaves were removed from the rooms the morning after freezing, and from this point on were handled exactly as those in Series 2.

Notes. Early in the season, when the wheat was two or three inches high, severe winds blew out the young plants at the north end of the plot very badly, this set-back resulting in an uneven ripening of the crop. The wheat at the protected south end of the plot was in flower before that at the north end had headed out. The first ten cuttings were made from the south end, while the remainder were made some from the north and some from the south end, but the wheat from the two sections was never mixed. The samples which were cut at the north end are marked by asterisks in all the tables. It will be noticed that this wheat is lower in grade, weight per bushel, total nitrogen, etc., than that obtained from the south end of the plot, but since the results are similar in all other respects they are reported together in the tables.

The material obtained by this artificial procedure is admittedly not in every way comparable to wheat which has been frozen under natural conditions, and did not make it possible to study the effect of allowing uncut frozen grain to stand in the field, but the samples are nevertheless of great value for studying the damage caused by frost under known and controlled conditions. Such a procedure permits a study of frost damage at all stages of maturity, every sample being grown under the same environmental conditions.

The samples in this series are of much greater value than those in Series 2, and while the milling and baking results are but casually referred to, the analytical

results are tabulated and discussed in some detail in a later section of the paper.

EXPERIMENTAL RESULTS

Series 1

The data concerned with this series are summarized in six tables. The percentage dry matter content at the time of cutting is included in each table in order to facilitate comparisons of the various fractions studied.

Fresh and dry weight. In Table 1 are presented the date of cutting, the number of days from flowering, the percentage dry matter, the weight per 1000 kernels and the weight of moisture in each sample at the time of cutting, and also the weight per 1000 air-dried kernels.

The period of development extended from July 21, the day of flowering, until Sept. 2 on which day the wheat was considered fully ready to cut with a binder. The collection period extended from July 26 to Sept. 17, the last cutting being made at least two weeks after the grain was considered ripe.

While kernel formation was still incomplete, the percentage dry matter in the wheat remained approximately constant at 26-27%. As soon as the kernels were fully

Table 1.

Fresh and dry weight of wheat kernels in relation to maturity at cutting

1	2	3	4	5	6	7	8
				Weight per thousand kernels			
Sample No.	Date of cutting	No. of days from flowering	Dry matter at time of cutting	Of fresh kernels		Of kernels dried at room temp.	Weight of moisture per 1000 fresh kernels
				Fresh	Dry		
			%	gm.	gm.	gm.	gm.
M 1	July 26	5	26.8	-	-	2.91	-
2	28	7	26.9	-	-	-	-
3	30	9	26.8	22.98	6.16	5.20	16.82
4	Aug. 1	11	26.0	30.62	7.96	6.90	22.66
5	4	14	32.0	36.63	11.72	10.45	26.18
6	6	16	35.7	41.71	14.89	13.27	28.44
7	8	18	40.2	43.98	17.68	16.24	27.74
8	11	21	44.6	46.70	20.83	19.97	26.73
9	13	23	48.9	49.35	24.15	23.43	25.20
10	15	25	50.2	53.48	26.85	25.67	26.62
11	19	29	53.2	55.68	29.63	29.31	26.05
12	22	32	55.7	56.53	31.47	31.31	25.06
13	25	35	58.4	54.33	31.71	32.00	22.62
14	28	38	59.8	54.23	32.41	31.98	21.82
15	30	40	70.4	48.22	33.94	32.44	14.28
16	Sept. 2	43	74.3	43.40	32.26	32.38	11.14
17	5	46	85.3	39.45	33.64	32.22	5.81
18	10	51	82.4	41.04	33.82	-	7.22
19	17	58	85.6	38.88	33.30	-	5.58

formed, however, it rose fairly regularly until the wheat was fully mature. There were irregularities in the rise, but these can be attributed largely to the changes in weather conditions during the development period. Figure 1 shows the percentage dry matter at cutting plotted against the number of days from flowering until the date of cutting. After the kernels had reached full size, the curve for the first five cuttings is straight. The curve for the last four or five cuttings is also approximately straight and parallel to the first section. The remainder of the curve has a much smaller slope than the other sections, but is also fairly straight when considered alone. During the first and last periods of development, the weather was bright and warm, but from Aug. 15 to Aug. 25 between which days the samples represented by the centre portion of the curve were cut, the weather was dark and cool with occasional showers.

Had the weather been uniform during the whole period, the curve resulting from the dry matter and days from flowering data would probably have been more nearly a straight line. Such a straight line has been drawn, and while it would probably never be obtained perfectly in any series, it does more nearly represent the probable course of development of the wheat had not the complicating factor of weather changes been operative.

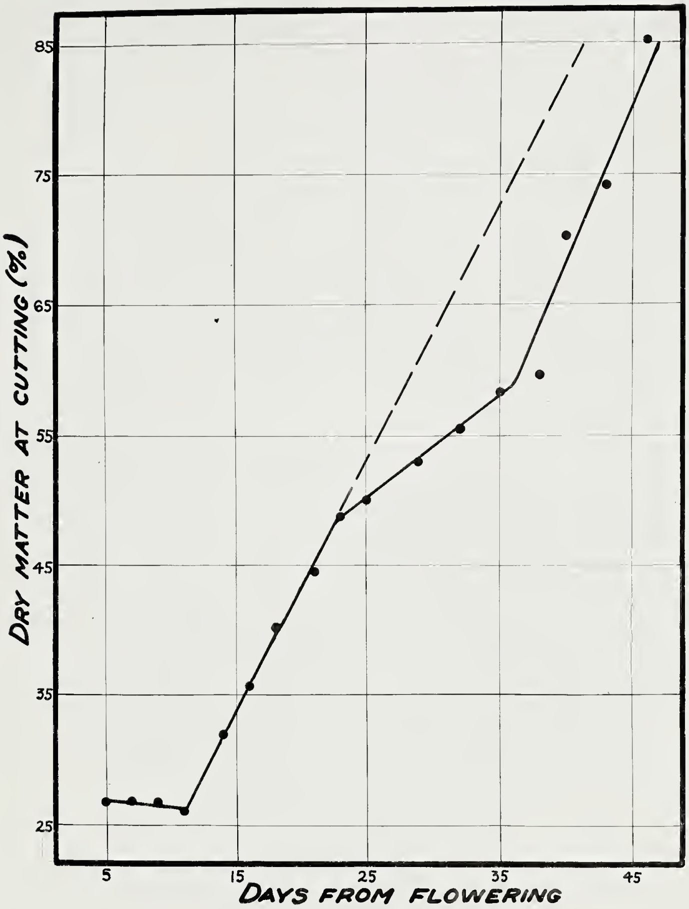

Fig. 1.

Percentage dry matter in wheat at time of cutting in relation to age of kernels.

That damp weather affects the dry matter content even after the wheat is dead ripe is shown by the results for samples M 17 to M 19. The first and last of these three were cut on bright warm days, while M 18 was cut on a damp morning. It contained 3% more moisture than M 17 or M 19.

The percentage moisture, of course, varied inversely as the percentage dry matter, but the total weight of water in the wheat was remarkably constant during a large proportion of the time in which the kernels were filling. From the time that the kernels were fully formed until they had reached a dry matter content of 55-60% the changes in weight of water were not more than 3 gm. or so per 1000 kernels, but as soon as this latter stage was reached rapid desiccation of the kernels begins. This fact is in agreement with the findings of Brenchley and Hall (12) and Woodman and Engledow (41), although the length of the constant-weight period more closely resembles that found by the former workers. It will become clear as the discussion in this paper proceeds that many other changes in the kernels ceased at this time, and that rapid desiccation is apparently directly related to, and possibly the cause of, this cessation. This is the most critical stage in the development period from the viewpoint of both the scientist and the farmer.

The figures for weight per 1000 fresh kernels show two main things: first, that the fresh weight increased steadily until rapid desiccation set in, and then decreased noticeably; and second, that by the same time the kernels had very nearly reached their maximum dry weight. The fresh weight rose slowly just before, and fell slowly just after the maximum had been reached, but both the rise and the fall were steady and continuous. The maximum dry weight was reached a week after rapid desiccation set in, but it is questionable whether any of the differences among the values for samples M 14 to M 19 were due to factors other than sampling errors. In any case, the changes in dry weight were very small after the grain reached a dry matter content of 56%, and the losses, if any, in allowing grain to stand in the field after it was fully ripe, were small.

Further valuable information is revealed by a comparison of the weight of dry matter per 1000 fresh kernels dried immediately at 98° C. with that of kernels allowed to air-dry in the laboratory atmosphere. First, these figures show that in every sample up to M 13 the dry weight of air-dried kernels was lower. From M 13 to M 19, the differences, although fairly great in one or two cases, are probably largely the result of experimental error. The results from these two groups afford a certain rough estimate of the amount of respiration which occurred

during the drying of the kernels, and points to the fact that under laboratory conditions, at least, respiration practically ceases when the grain reaches a dry matter content of 56%. Figures 2 and 3 present these results in graphical form, and it is readily seen that the actual losses due to respiration were fairly constant regardless of the dry matter content at the time of cutting, but that the percentage of the total weight of the kernel lost in respiration steadily decreased. This probably means that the embryo was fully formed fairly early in the development of the wheat, and that it functioned in respiratory actions just as rapidly in the very immature kernel as in the more mature. Furthermore, the dry weight per 1000 air-dried kernels reveals the fact that even when the grain was threshed out at cutting, the loss in weight was very slight, if any, provided that the wheat contained approximately 56% or more dry matter at the time it was cut. This is again significant from the farmer's viewpoint, because any grain cut after the kernels have become pink and the dough fairly firm, will give practically as high a yield as could be expected from the same wheat were it allowed to become dead ripe before cutting.

Total nitrogen. The results of all the total nitrogen determinations are summarized in Table 2 where they are presented as percentages of the dry matter and as actual weight per 1000 kernels.

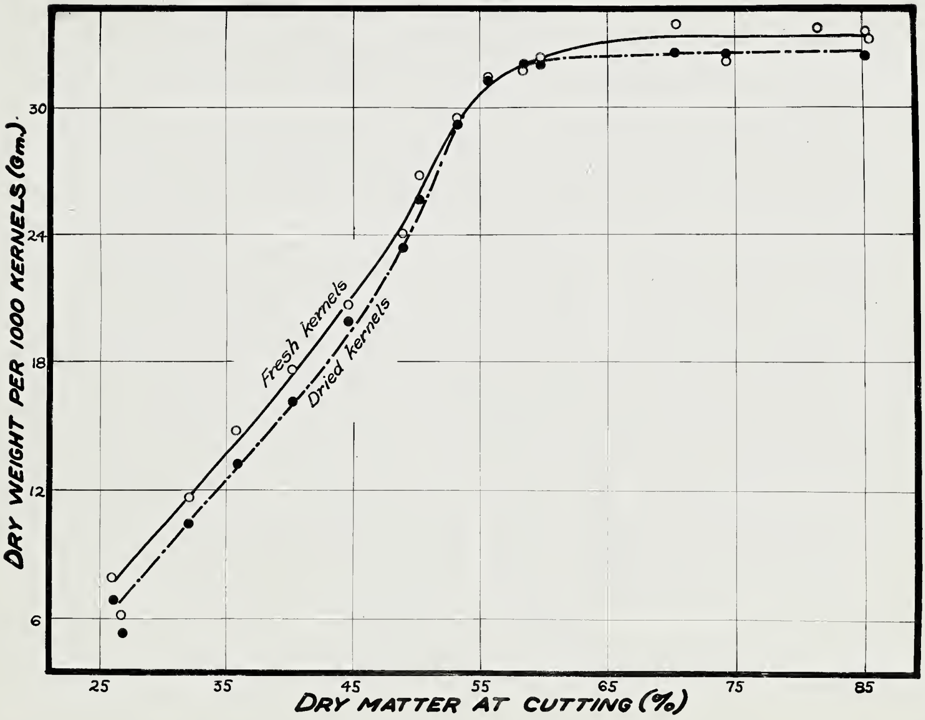

Fig. 2.

Dry weight of kernels in relation to stage of maturity at time of cutting.

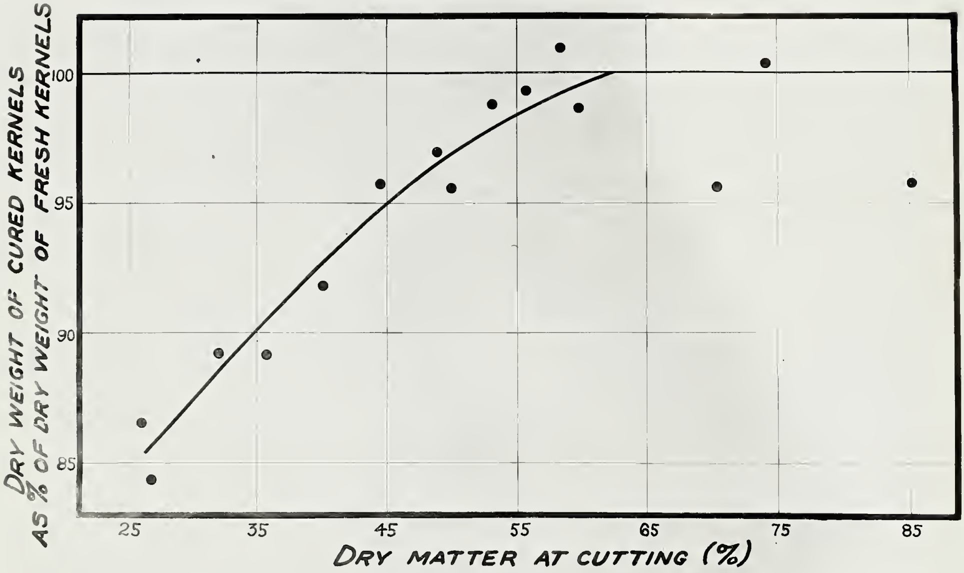

Fig. 3.

Percentage weight relations between cured and fresh kernels at different stages of maturity.

Table 2.

Total nitrogen in wheat kernels in relation to maturity at cutting and method of drying

1	2	3	4	5	6	7	8
Sample No.	Dry matter at time of cutting	In fresh kernels			In kernels dried at R.T.		In kernels dried at 60°C. for 12 hours. As % of dry weight
		As % of fresh weight	As % of dry weight	As wt. per thousand	As % of dry weight	As wt. per thousand	
	%	%	%	gm.	%	gm.	
M 1	26.8	0.67	2.51	-	-	-	2.57
2	26.9	0.67	2.50	-	-	-	2.52
3	26.8	0.68	2.54	-	3.01	0.156	2.56
4	26.0	0.73	2.80	-	2.98	0.206	2.58
5	32.0	0.79	2.47	-	2.77	0.289	2.55
6	35.7	0.88	2.46	-	2.76	0.366	2.60
7	40.2	1.00	2.48	-	2.70	0.438	2.54
8	44.6	1.13	2.54	-	2.65	0.529	2.56
9	48.9	1.22	2.50	0.604	2.64	0.618	2.61
10	50.2	1.31	2.61	0.701	2.69	0.690	2.65
11	53.2	1.40	2.63	0.779	2.76	0.809	2.75
12	55.7	1.58	2.83	0.890	2.98	0.933	2.87
13	58.4	1.68	2.88	0.913	3.00	0.960	2.99
14	59.8	1.73	2.89	0.937	3.02	0.966	2.96
15	70.4	2.07	2.94	0.998	2.98	0.967	2.98
16	74.3	2.13	2.87	0.926	2.96	0.958	3.00
17	85.3	2.52	2.95	0.992	3.01	0.969	2.97
18	82.4	2.53	3.07	1.038	-	-	-
19	85.6	2.58	3.02	1.006	-	-	-

The percentage total nitrogen in fresh kernels remained about constant while the kernels were forming, but from the time they were fully formed until the wheat reached maturity there was a gradual but constant rise.

The same values expressed on the basis of percentage nitrogen in dry matter, however, show a much different relation. Although there were slight variations, and a slight downward trend in the first six cuttings (the value for M 4 is obviously in error) this decrease is small, in fact hardly large enough to be significant (Fig. 4). From the seventh cutting until maturity the percentage nitrogen in the wheat increased, this fact being in agreement with the larger part of the work cited from the literature, and also in agreement with the idea that a rise in percentage nitrogen must occur during the later stages of development owing to loss of carbohydrates by respiration. The rise found in the percentage nitrogen in the samples of this series may be almost entirely explained if we accept the figures of McGinnus and Taylor (22) for the loss of carbohydrates by the respiration of threshed kernels as applicable to kernels in plants ripening in the field.

The slight variations in the percentages nitrogen of the first nine samples cut are not in strict agreement with the results of some other workers, who have found

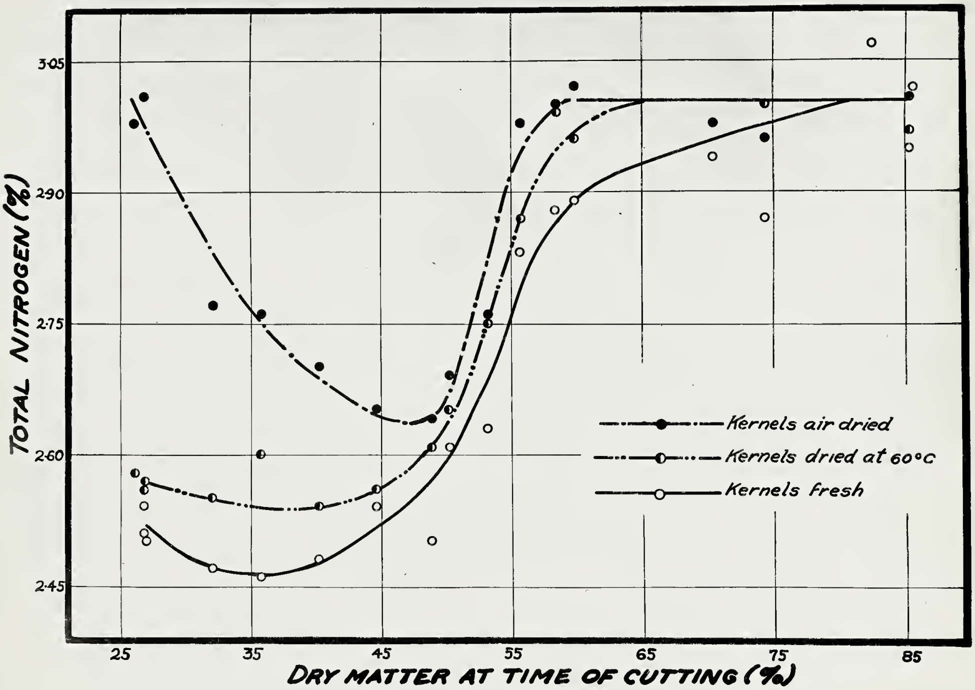

Fig. 4.

Percentage total nitrogen in wheat in relation to stage of maturity and method of drying.

first a decided decrease and then an increase as already noted. A slight tendency toward this decrease is noticeable, and is very similar to, though slightly less than, that found by Woodman and Engledow (41). The greatest spread between the original percentage nitrogen and the minimum is only 0.08%, which, while greater than the experimental error, is not very significant. Such a slight decrease might be the result of the gradual filling in of the endosperm which is slightly lower in total nitrogen than the bran layers. This explanation has been offered by many others, and while it has undoubtedly been overworked, it may logically explain the slight decreases noted in the values for these early samples.

The percentage total nitrogen in air-dried kernels shows a different trend, (Fig. 4). The earliest sample tested was as high as the latest, and the whole series, except for the mature samples, was higher in this respect than the fresh material. It has already been noted that the dry weight of the air-dried kernels was lower than that of the fresh kernels, owing to respiration during drying. This loss in weight was caused by loss of carbohydrates and is sufficient to explain the higher percentages total nitrogen in the air-dried samples. In this case, the decrease in percentage nitrogen from sample M 3 to M 9 cannot be due entirely to changes in the bran-endosperm ratio, since it is more pronounced than in

the fresh kernels. It is probably due largely to the greater loss of carbohydrates in the more immature samples during air-drying.

After the minimum percentage of nitrogen had been reached in sample M 9, there was a decided increase in all succeeding samples until the wheat had reached a dry matter content of 55.7%. Obviously respiration had been active until this stage was reached. The percentage total nitrogen in the samples cut after this stage was constant and equal to that of the mature samples analysed fresh. From these results, and from the curves in Fig. 4, it seems that while respiration of kernels in the laboratory ceases when the wheat reaches a dry matter content of 56%, the wheat standing in the field continues to respire until the dry matter increases to 75%. In any case, it is apparent that whether wheat is cut or allowed to stand uncut after it reaches the critical stage of development, the total nitrogen percentage will reach the same level.

The total nitrogen percentages in kernels which were dried rapidly at 60° C. were similar for all those samples cut after rapid desiccation set in. The percentage in each sample up to M 12 was higher than that for the fresh material and lower than that for the air-dried samples. This intermediate relationship, easily noted in Fig. 4, can be explained by the fact that while rapid

drying was in progress respiration losses would be high, but the short period of drying did not allow for as great a total loss of carbohydrates as was possible when the grain dried slowly at room temperature.

Turning now to a consideration of the total weight of nitrogen per 1000 kernels, it is obvious that there was a steady increase during the first 35 days of development, but that by the thirty-fifth day, on which the dry matter content was 58.4%, practically all of the nitrogenous material had been accumulated in the kernel. The amounts of total nitrogen in fresh and air-dried material should have been the same provided no losses of nitrogen occurred during respiration. There are rather wide discrepancies between some of the pairs of values in columns 5 and 7 (Table 2), but these values are of the same order, and when it is considered that those in column 5 were calculated from the analyses of fresh moist kernels, while those in column 7 are from dried, well ground and carefully sampled material, the agreement must be considered fair, as the first method of determining the amounts of total nitrogen is subject to much greater possibility of error than the second.

Since the figures for the air-dried kernels are the more reliable, it is safe to say that the translocation of nitrogenous material ceased when the wheat reached a dry matter content of 60%. Taken in

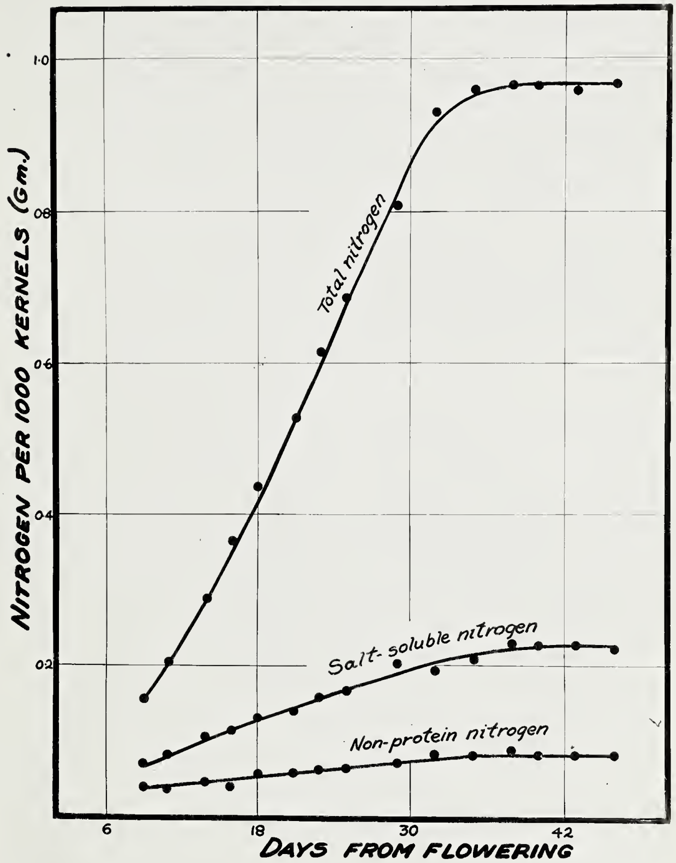

Fig. 5.

Weight of nitrogen in wheat in relation to age of kernels.

conjunction with the results for weight of dry matter per 1000 kernels of wheat (Fig. 6), these results prove three main things: first, that there will be little loss in yield of crop or protein although the wheat be cut while it contains 40% moisture, an amount considerably higher than grain usually contains at the time of harvest; second, that losses, if any, resulting from delayed harvesting after the grain is fully ripe, are small; and third, that it is unreasonable to suppose that a higher percentage protein sample can be obtained by cutting wheat early than would be secured by allowing it to mature.

Ammonia nitrogen. The analytical results for volatile nitrogen, which for lack of a clearer knowledge of its nature, has been termed ammonia, are summarized in Table 3. From a consideration of the method of analysis it is obvious that this fraction is free in the kernel, and from the small and changing amounts present, that it is transitory in nature. Nevertheless, the quantities showed a regular relationship which is the more significant since in dried material no measurable amount of this fraction could be detected.

This fraction may not contain compounds which are the starting point of nitrogenous synthesis, but its nature and its presence in such small quantities might justify this belief. A fairly definite hypothesis which takes into consideration all the analytical results is here presented.

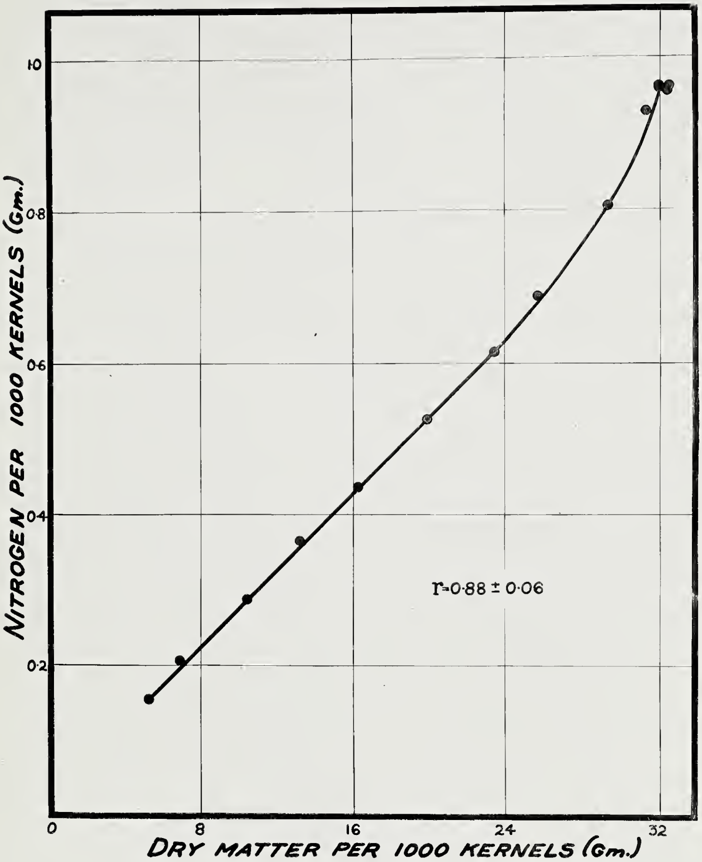

Fig. 6.

Weight of total nitrogen in relation to weight of dry matter in wheat kernels.

Table 3.

Ammonia nitrogen in wheat kernels in relation to maturity at cutting

1	2	3	4
Sample No.	Dry matter content at cutting	Ammonia N. as % of total N.	Ammonia N. as weight per 1000 kernels
	%	%	mg.
N 1	26.8	0.67	-
2	26.9	0.61	-
3	26.8	1.18	1.84
4	26.0	1.28	2.63
5	32.0	1.34	3.87
6	35.7	1.18	4.32
7	40.2	1.09	4.77
8	44.6	0.87	4.60
9	48.9	0.60	3.71
10	50.2	0.56	3.86
11	53.2	0.30	2.43
12	55.7	0.25	2.33
13	58.4	0.04	0.38

While the kernel is forming, a certain amount of this ammonia nitrogen accumulates in the watery fluid making up the kernel contents. Until the kernel is fully formed, little, if any of this material is utilized in synthesis, and hence its amount increases both in percentage and weight. Once the kernel reaches full formation active synthesis of higher compounds begins. These are non-volatile. The ammonia nitrogen expressed as a percentage at once ceases to increase, owing to its utilization, but the actual weight increases for a few days, indicating that utilization is hardly as great as translocation. Later both percentage and total weight decrease as the utilization of the ammonia in synthesis becomes greater than its supply by translocation. After the wheat passes a dry matter content of 56%, this fraction disappears as far as can be detected by the method of analysis. Since it does not appear in the dried samples it seems reasonable to attribute its presence in the fresh material to a direct relation to metabolic processes. Furthermore, these results substantiate the contention that little, if any, translocation of nitrogenous materials into the kernel occurs after the wheat contains more than 58% dry matter.

Salt-soluble nitrogen. The results of the salt-soluble nitrogen determinations are presented in Table 4. The amounts of this fraction are expressed in two ways,

Table 4.

Salt-soluble nitrogen in wheat kernels in relation to maturity at cutting and method of drying

1	2	3	4	5	6	7
		In fresh kernels		In kernels dried at room temp.		
Sample No.	Dry matter content at cutting	As % of total nitrogen	As weight per thousand	As % of total nitrogen	As weight per thousand	In kernels dried at 60° for 12 hr. As % of total nitrogen
	%	%	gm.	%	gm.	%
M 1	26.8	74.1	-	-	-	57.6
2	26.9	72.0	-	-	-	57.9
3	26.8	74.0	0.116	44.5	0.070	51.2
4	26.0	68.6	0.153	39.3	0.081	42.6
5	32.0	58.3	0.169	37.2	0.108	35.3
6	35.7	51.2	0.188	31.2	0.114	31.2
7	40.2	44.3	0.194	29.6	0.130	23.6
8	44.6	35.6	0.187	26.4	0.140	21.9
9	48.9	32.0	0.193	25.4	0.157	19.2
10	50.2	28.7	0.204	24.2	0.167	20.0
11	53.2	25.1	0.196	25.4	0.205	21.1
12	55.7	23.3	0.208	20.8	0.194	18.8
13	58.4	21.5	0.197	22.0	0.211	18.7
14	59.8	22.0	0.207	23.9	0.230	18.9
15	70.4	18.3	0.183	23.5	0.227	22.2
16	74.3	19.7	0.181	23.6	0.226	20.7
17	85.3	19.7	0.195	22.8	0.222	22.6
18	82.4	19.9	0.206	-	-	-
19	85.6	23.2	0.233	-	-	-

first as a percentage of the total nitrogen and second as actual weight per 1000 kernels.

During the formation of the kernels the percentage salt-soluble nitrogen in the fresh material changed very little, but from the time of complete formation of the kernels until the time they contained 56% dry matter there was a decided drop in this percentage. From the results for this group it would appear that this drop continued for the soluble nitrogen in the wheat secured at the next four cuttings, and that this drop was followed by a gradual rise until the grain was fully mature. It must be pointed out, however, that with this fresh material it was very difficult to get a complete extraction of the soluble nitrogen in the more mature samples because, while the grain was too moist to be ground in a mill it was very hard to grind effectively in a mortar. When the values for soluble nitrogen in the air-dried kernels and the curves in Figure 7 are considered, it is obvious that the values for fresh samples M 15 to M 18 are lower than they should be, and that a constant level of 22 to 23% salt-soluble nitrogen was maintained from the time of cutting sample M 12 until the final days of the experiment.

The amounts of this fraction found in air-dried kernels are all very much lower than those found in corresponding fresh material in all samples which contained

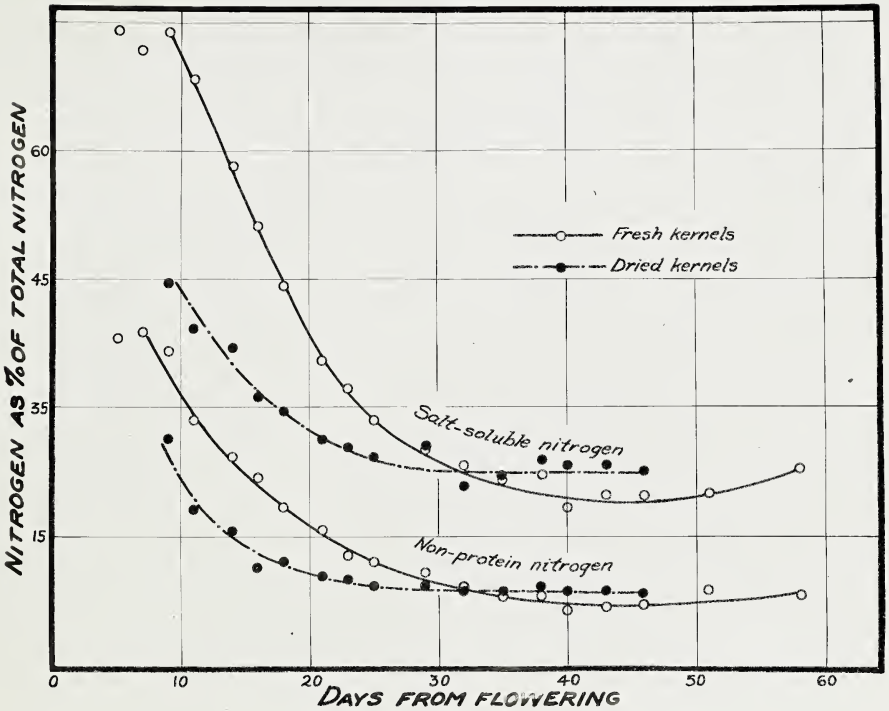

Fig. 7.

Percentage nitrogen fractions in wheat in relation to age of kernels.

less than 53% dry matter when they were cut. This shows that while the wheat is drying, even with translocation precluded, a large amount of this soluble nitrogen is utilized in the synthesis of more insoluble compounds, but that whether the wheat dries in the field or in the laboratory, no further synthesis occurs after the wheat reaches a dry matter content of 56% or thereabout.

These conclusions are substantiated by those for the wheat dried at 60° C., which at first fell into an intermediate position, but reached the same final value as in the fresh and air-dried kernels. The time available for synthesis during drying at this high temperature was very short, but actual synthesis was probably speeded up. The results for samples M 5 to M 14 are lower than for the air-dried material and suggest that either synthesis was very rapid during drying or else this heat caused denaturation of some of the compounds normally extracted by the salt solution. Which of these explanations is correct is indeterminable from the results.

The weight of salt-soluble nitrogen in the kernels increased slowly until 35 days after flowering (Fig. 5) or until the dry matter content reached 58%, after which it remained about constant. The increases, however, were very small when compared with those for total nitrogen, and show that almost as rapidly as these

compounds were produced in the kernel either by translocation or primary synthesis, they were utilized in the building up of higher compounds. This fraction, of course, included such compounds as globulin and albumen which are themselves the end-products of metabolism, but a certain proportion of the fraction was no doubt made up of materials potentially available for synthesis of the more characteristic gluten proteins. The fact that the salt-soluble nitrogen reached a constant value toward the end of the development period does not mean that this constant value is a measure of the amount of soluble protein present, for it will be seen in the consideration of non-protein nitrogen that the amount of this fraction too reaches a constant level in the mature wheat.

The amounts of soluble nitrogen present in air-dried kernels was less than that in the fresh material during the earlier stages of development, or until the wheat had reached a dry matter content of 56%. The weight of salt-soluble nitrogen extracted from the more mature samples reached a constant level which was maintained from sample M 14 to M 17. Incomplete extraction in the case of the fresh kernels probably accounts for the discrepancies between the pairs of values for fresh and dried material, and it is apparent that 1000 kernels of the ripe wheat contained about 0.225 gm. of nitrogen which was soluble in 5% K_2SO_4.

Non-protein nitrogen. The analytical data concerning non-protein nitrogen is presented in Table 5, in which the individual results are expressed in three ways: first, as a percentage of the total nitrogen; second, as a percentage of the salt-soluble nitrogen; and third, as weight per 1000 kernels.

Only the lower nitrogen compounds make up this fraction, and it is seen that even the mature wheat contained 8 to 9% of its total nitrogen in this form, no matter how drying was carried out. The relations existing between the percentage and weight results for fresh and air-dried kernels are very similar to those existing between the results for salt-soluble nitrogen in the same groups, and are probably explicable by the same reasoning.

The non-protein expressed as a percentage of salt-soluble nitrogen showed a tendency toward a decrease as development progressed. This simply means that while the non-protein fraction of the salt-soluble nitrogen was gradually utilized in synthesis of higher compounds, the protein fraction suffered less change, and hence gradually predominated in the soluble material.

The amounts of non-protein nitrogen present in the rapidly dried material, however, are very different from those found in either fresh or air-dried kernels. In every sample from M 1 to M 14 the values were higher than the corresponding values for either of the other groups.

Table 5.

Non-protein nitrogen in wheat kernels in relation to maturity at cutting and method of drying

1	2	3	4	5	6	7	8	9
Sample No.	Dry matter at time of cutting	In fresh kernels			In kernels dried at room temperature			In kernels dried at 60°C. for 12 hours. As % of total N.
		As % of total N.	As % of salt-soluble N.	As weight per thousand	As % of total N	As % of salt-soluble N.	As weight per thousand	
	%	%	%	gm.	%	%	gm.	%
M 1	26.8	38.2	51.5	-	-	-	-	50.6
2	26.9	38.8	53.9	-	-	-	-	51.6
3	26.8	36.6	49.5	0.057	26.6	59.8	0.042	46.9
4	26.0	28.6	39.1	0.064	18.1	46.1	0.037	37.2
5	32.0	24.3	41.7	0.070	15.9	42.7	0.046	30.6
6	35.7	22.0	43.0	0.080	11.6	37.2	0.042	22.3
7	40.2	18.5	41.8	0.081	12.2	41.2	0.054	20.5
8	44.6	15.8	44.4	0.083	10.6	40.2	0.056	17.2
9	48.9	13.0	40.6	0.077	10.2	40.2	0.063	13.8
10	50.2	12.1	42.2	0.083	9.3	38.4	0.064	13.6
11	53.2	10.9	43.4	0.083	9.4	37.0	0.076	11.6
12	55.7	9.2	39.5	0.082	9.1	43.8	0.084	10.5
13	58.4	8.0	37.2	0.073	8.7	39.5	0.083	9.7
14	59.8	8.1	36.8	0.078	9.3	38.9	0.090	9.5
15	70.4	6.5	35.5	0.064	8.7	37.0	0.084	8.7
16	74.3	6.9	35.0	0.064	8.8	37.3	0.084	8.0
17	85.3	7.1	36.0	0.071	8.6	37.7	0.084	8.8
18	82.4	8.8	44.2	0.088	-	-	-	-
19	85.6	8.5	36.6	0.086	-	-	-	-

This large increase in percentage non-protein nitrogen was undoubtedly brought about by heat effects, but whether the heat was the direct cause of the changes or whether these were due to the increased activity of enzymes cannot be determined without further study.

It is apparent that whatever the cause of the increases may have been, the effect is noticeable only in that material which was extractable by the salt solution. The nature of this fraction must have been radically changed, and some of the higher compounds split to forms not precipitated by trichloracetic acid.

Other nitrogen fractions. The data presented in Table 6 concerns that fraction of nitrogen precipitated from the salt-soluble extract by trichloracetic acid, and water-soluble nitrogen. The figures for precipitated nitrogen have already been dealt with indirectly in the consideration of salt-soluble and non-protein fractions. The most outstanding results are those for the material dried at 60° C., and these have been mentioned in the last paragraph. The final values for all three groups were fairly similar, as were the individual values for the mature samples of the air-dried group, these results indicating that very little, if any, change took place after desiccation of the kernels began.

Table 6.

Nitrogen ppt. by trichloracetic acid, and water-soluble nitrogen in wheat kernels in relation to maturity at cutting and method of drying

1	2	3	4	5	6	7
Sample No.	Dry matter content at cutting	Nitrogen ppt. by trichloracetic acid			Water-soluble nitrogen	
		In fresh kernels. As % of total N.	In kernels dried at room temp. As % of total N.	In kernels dried at 60° C. As % of total N.	In kernels dried at room temp. As % of total N.	In kernels dried at 60°C. As % of total N.
	%	%	%	%	%	%
M 1	26.8	35.9	-	7.0	-	-
2	26.9	33.2	-	6.3	-	-
3	26.8	37.2	17.9	4.3	-	-
4	26.0	40.0	21.2	5.4	-	40.3
5	32.0	34.0	21.3	4.7	28.5	34.1
6	35.7	29.2	19.6	8.9	23.9	28.5
7	40.2	25.8	17.4	3.1	23.0	24.4
8	44.6	19.8	15.8	4.7	20.4	21.1
9	48.9	19.0	15.2	5.4	20.5	17.6
10	50.2	16.6	14.9	6.4	18.6	17.7
11	53.2	14.2	16.0	9.5	18.8	17.1
12	55.7	14.1	11.7	8.3	16.8	15.5
13	58.4	13.5	13.3	9.0	17.3	13.7
14	59.8	13.9	14.6	9.4	17.2	13.7
15	70.4	11.8	14.8	13.5	17.4	14.6
16	74.3	12.8	14.8	12.7	16.9	14.3
17	85.3	12.6	14.2	13.8	16.6	15.2
18	82.4	11.1	-	-	15.6	-
19	85.6	14.7	-	-	14.6	-

The water-soluble nitrogen results ran almost parallel with those for salt-soluble nitrogen, although they were considerably lower. This fraction contained all the non-protein nitrogen, and some of the lower synthetic products which were extracted by the salt solution.

Conclusions. 1. The percentage dry matter in wheat remains constant during kernel formation, and then, subject to modifications due to weather conditions, rises regularly until harvest.

2. The weight of water in the kernels is remarkably constant from the time they are formed until the dry matter content of the wheat reaches 56%. At this point rapid desiccation of the grain sets in.

3. The fresh weight of the wheat rises until the time of desiccation, then falls until the grain is mature. The dry weight rises to a fairly constant level at this time, after which changes, if any, are very slight.

4. The percentage total nitrogen in fresh kernels at first drops very slightly, then rises gradually until the grain is fairly ripe. The percentage in air-dried kernels (normal) is high in the early samples owing to respiration during drying, falls gradually for a few days, then rises again to a constant high level in all wheat cut after the dry matter content has reached 56%.

5. There is a very small volatile nitrogen fraction present in fresh kernels. This probably consists of compounds utilized in primary synthesis.

6. The percentage of the lower nitrogen fractions found in the kernels decreases from the time the kernels are formed until they contain 56% dry matter, then remains constant; but the actual amount of these fractions increases slightly in all but the more mature samples.

7. There is a critical stage in the development of wheat, which is coincident with, and possibly closely related to, the beginning of rapid desiccation of the kernels. Once this stage has been reached, all changes in kernel composition practically cease.

8. Wheat can be cut any time after it contains 58% dry matter, and the losses in yield of grain or protein, if any, will be small.

Series 2

General. It has already been stated that this series was of little value in the study of frozen wheat because the frost treatment to which the samples were subjected was too light to cause measurable damage. None of the analytical results will be included here, because

their volume greatly exceeds their value. While the series was a disappointment from the standpoint of a study of frost injury, the results obtained for the unfrozen checks give valuable evidence in substantiation of the data discussed in the last section.

The total nitrogen results agree very well with those obtained from Series 1 material, although the Renfrew wheat appeared to be constant in percentage total nitrogen from the immature to ripe stages.

The results of this series also showed that relatively severe frost for short periods of time may not injure the wheat under these artificial conditions. The range in temperatures used in this series was from 30° to 18° F., but the only way in which the frozen samples differed materially from the checks was in grade. Probably the effects of this short duration of frost were confined to the bran layers.

Glutenin determination. The determination of glutenin in flour has been a standard procedure practised by many cereal chemists. Some of the results obtained from this series led to the belief that the methods used in this determination were not satisfactory. Further study was carried out and as a result of this study together with other work concerning the nature of wheat proteins, the glutenin determination has been discontinued at this laboratory.

The routine method which had been used for some time at this university was that described by Blish and his co-workers (7). When this method was used to determine the glutenin in the flours milled from the unfrozen check wheats, it was found that there was a tendency for the immature samples to yield flours higher in glutenin than were the flours from mature wheat, despite the fact that the total nitrogen in the latter flours was in every case as high or higher than in that from immature material.

It was decided to compare the results obtained by this method with those obtained by using the two other methods for this determination as described by Blish and Sandstedt (8) and Sharp and Gortner (29). The analytical results obtained from a study of flour from Reward and Renfrew wheat are presented in Table 7.

The lowest numbered sample in each case was the most immature. It will be noted that the results by the three methods agreed very poorly for the immature samples, although those for mature material obtained by the first and third methods agreed fairly well. The results by Sharp and Gortner's method were all high.

It has never been found possible to show a definite relation between glutenin as determined by any of these methods and flour strength or quality. The value of the determination has never been established, and since

Table 7.

Percentage glutenin in flour in relation to stage of maturity and method of analysis

1	2	3	4
Sample	Blish, Abbott and Platenius ($Ba(OH)_2$ method	Sharp and Gortner (70% alcohol & sand)	Blish & Sandstedt (direct method)
Reward			
A.F. 27	8.3	8.0	8.9
30	8.9	9.2	9.0
33	7.6	8.3	8.7
36	7.8	8.5	8.4
39	8.3	9.2	8.3
42	7.9	8.9	8.4
45	7.7	9.0	7.9
48	7.9	-	8.0
Renfrew			
A.F. 78	5.8	6.3	
81	5.5	6.1	
84	5.4	6.9	
87	5.2	7.1	
90	5.2	6.7	
93	5.1	6.8	
96	5.1	6.9	
99	4.8	6.5	
102	4.8	6.5	

such conflicting results have been obtained it would appear that the uncertainty of the methods justifies the discontinuation of the determination at the present time. This discontinuation is further justified by the report of Blish (6) to the Association of Official Agricultural Chemists and by work done by Cook (13) which question the existence of so-called glutenin as a separate and distinct fraction in the wheat.

For these reasons, this part of the work has been dropped, and no conclusions are drawn from the data in Table 7.

Series 3

General. The experimental results from the samples in this series while presented in detail, will not receive the same individual consideration as those in Series 1. There are 79 samples on which the various determinations were made, and the accumulated data are too voluminous to permit of reference to more than general trends and group results in most instances.

It is necessary to bear in mind that the samples are not all completely comparable as to growth conditions. Those collected at the north end of the field, and which were set back by soil blowing early in the season, are designated in the tables. In all these except Table 8, horizontal comparisons show frost effects, and vertical

Table 8.

Dry matter content and frost treatment of individual samples

1	2	3	4	1	2	3	4
Sample No.	Dry matter content at cutting	Frost treatment		Sample No.	Dry matter content at cutting	Frost treatment	
		Amount	Time held at min. temp.			Amount	Time held at min. temp.
	%	°F.	hr.		%	°F.	hr.
1 a	31.2	8	4	9 a	46.8	8	4
b	31.2	4	4	b	46.8	4	4
c	31.2	-	-	c	46.8	-	-
2 a	34.0	13.5	4	10 a	46.7	14	4
b	34.0	9	4	b	46.7	10	4
c	34.0	-	-	c	46.7	-	-
3 a	34.9	8	4	11 a*	48.3	8	4
b	34.9	4	4	b*	48.3	4	4
c	34.9	-	-	c*	48.3	-	-
4 a	38.1	14	4	12 a*	50.5	14	4
b	38.1	10	4	b*	50.5	10	4
c	38.1	-	-	c*	50.5	-	-
5 a	39.4	8	4	13 a*	50.5	8	4
b	39.4	4	4	b*	50.5	4	4
c	39.4	-	-	c*	50.5	-	-
6 a	40.9	14	3.5	14 a*	51.1	14	4
b	40.9	10	3.5	b*	51.1	7	4
c	40.9	-	-	c*	51.1	-	-
7 a	43.8	8	4	15 a*	51.3	8	4
b	43.8	4	4	b*	51.3	4	4
c	43.8	-	-	c*	51.3	-	-
8 a	45.5	14	4	16 a*	53.3	14	4
b	45.5	10	4	b*	53.3	10	4
c	45.5	-	-	c*	53.3	-	-

* Samples from north end of the field.

Table 8 (continued).

1	2	3	4
Sample No.	Dry matter content at cutting	Frost treatment	
		Amount	Time held at min. temp.
	%	°F.	hr.
17 a	54.9	14	4
b	54.9	10	4
c	54.9	-	-
18 a*	55.8	8	3.5
b*	55.8	3	3.5
c*	55.8	-	3.5
19 a	56.8	8	4
b	56.8	4	4
c	56.8	-	-
20 a	57.3	8	4.
b	57.3	4	4
c	57.3	-	-
21 a*	57.4	14	4
b*	57.4	10	4
c*	57.4	-	-
22 a*	58.1	14	3.5
b*	58.1	7.5	3.5
c*	58.1	-	-
23 a	58.5	14	4
b	58.5	10	4
c	58.5	-	-
24 a*	59.1	14	3
b*	59.1	8	3
c*	59.1	-	-
25 a*	65.5	14	3.5
b*	65.5	7	3.5
c*	65.5	-	-
26 a	65.6	14	4
b	65.6	7	4
c	65.6	-	-
27	69.4	-	-

* Samples from the north end of the field. The difference between the two sections is described on page 24.

comparisons the effects of progressive development. The horizontal comparisons are the more valuable in this series, and to have tabulated separately the results of the north and south samples would have unnecessarily broken the continuity.

Production details: The details presented in Table 8 summarize the stage of maturity and frost treatment of the various samples. While most of the information is included in the other tables, this summary will facilitate comparisons and indicate all minor variations from the standard freezing conditions.

Grade. The data on grade (Table 9) show three main things: first, the progressive improvement as the wheat ripens; second, the influence of lack of uniformity and presence of green kernels; and third, the effect of frost.

The most immature check samples graded Feed, but from the time the wheat contained 40% dry matter until it reached 58 to 60% at cutting, the grade gradually improved. For samples in which the rapid desiccation, described in Series 1, set in before the wheat was cut, the grade of the uniform wheat from the south end of the plot was in every case either No. 1 or No. 2 Northern. Those samples from the north end, however, contained green kernels and lacked uniformity, and as a result, the mature check samples graded No. 3 and in one case No. 4 Northern. It appears that practically all grade changes take place during the relatively

short period while the wheat is increasing in dry matter from 40 to 60%; that all samples cut in a less mature condition will grade feed; that those allowed to ripen in the field will not improve in grade after the wheat has reached 60% dry matter; and that the presence of relatively few small green kernels causes a distinct cut in the grade.

The grade of frozen wheat, even where frost treatment was light (4°), except in two cases was lower than that of the check samples. Samples which were subjected to 8°, 10°, and 14° F. frost were nearly all lowered in grade and by approximately the same amount. The lowering of the grade by freezing mature wheat is of great practical importance. It will be shown that frost does not affect the composition of wheat providing it has reached a dry matter content of 58% before it is frozen, but the grade on such wheat is markedly affected. The milling and baking results, which are not included in this paper, show that the quality of the mature wheat is not reduced by frost, and hence the effect on the grade raises one of the great problems of equitable marketing of the wheat. Every indication supports the belief that, were it possible to tell at what stage of development the wheat was frozen, much of the frozen grain now grade No. 4 and No. 5, and even a little of that graded No. 6 might be included in the higher grades with no loss to the miller and baker, and great gain to the farmer. Since it is almost impossible

to ascertain the stage of development at which the grain was frozen it is difficult to grade the wheat fairly, but undoubtedly a lot of grain which makes first class flour and bread is graded No. 4 and No. 5 and some even No. 6 at the present time.

Germination. The germination results (Table 9) agree very well with those cited from the literature. The immaturity of the wheat at cutting appears to have no effect on its immediate germinating ability as in only one of the 27 check samples did less than 92% of the kernels germinate, and the results for the most immature samples were at least as high as those for the mature.

Frost injury to germination depends on the stage of development of the wheat and the severity of the frost to which it is subjected. The 4° frost did not reduce germination appreciably, even in the most immature samples. The 8° frost caused no damage to kernels containing over 56% dry matter. Injury to the germination of the samples subjected to 10° and 14° frost ceased when the dry matter content reached 59%.

Weight per measured bushel. The weight per bushel of each of these samples was determined twice, the first time immediately after threshing, and the second time after the wheat had been stored in a cool room for about 4 months. The figures presented in Table 9 are those for the latter determination, and while the samples varied

Table 9.

Grade, germination and weight of wheat in relation to stage of maturity and exposure to frost

1	2	3	4	5	6	7	8	9	10	11	12	13
Dry matter at time of cutting	Grade			Germination			Weight per measured bushel			Weight per 1000 kernels (13.5% moisture)		
	8° frost	4° frost	Check	8° frost	4° frost	Check	8° frost	4° frost	Check	8° frost	4° frost	Check
%				%	%	%	lb.	lb.	lb.	gm.	gm.	gm.
31.2	Feed	Feed	Feed	71	97	95	43.0	45.0	43.0	13.50	13.59	12.86
34.9	Feed	Feed	Feed	73	95	98	46.5	49.5	49.5	16.58	18.18	16.99
39.4	Feed	Feed	Feed	76	95	99	50.5	52.0	53.0	19.75	20.55	19.55
43.9	Feed	5	4	83	95	96	55.5	57.0	57.5	23.38	24.28	23.77
46.8	Feed	5	4	90	96	96	57.5	57.5	58.0	26.13	25.64	25.89
48.3*	Feed	6	6	90	87	96	56.5	55.0	56.5	23.89	26.46	26.29
50.5*	Feed	Feed	5	80	90	98	55.5	57.0	59.5	26.47	28.91	28.43
51.1*	Feed	-	5	80	--	96	57.5	--	60.0	26.92	--	29.56
51.3*	Feed	6	5	88	95	94	58.5	58.5	58.5	29.21	28.05	27.12
55.8*	Feed	Feed	4	83	91	92	61.5	61.5	62.5	31.53	32.13	32.70
56.8	6	4	3	96	95	96	62.0	63.5	64.5	35.34	35.94	35.29
57.3	6	4	1	90	97	81	63.0	64.0	65.0	37.68	36.01	36.43
58.1*	5	-	3	96	--	95	61.0	--	62.0	35.10	--	35.50
59.1*	6	-	4	97	--	96	62.0	--	64.0	36.11	--	35.55
65.5*	3	-	3	95	--	92	63.5	--	63.5	35.97	--	33.18
65.6	5	-	2	94	--	95	62.5	--	64.5	36.96	--	36.96

* Samples from north end of the field.

Table 9 (continued).

1	2	3	4	5	6	7	8	9	10	11	12	13
Dry matter at time of cutting	Grade			Germination			Weight per measured bushel			Weight per 1000 kernels (13.5% moisture)		
	14° frost	10° frost	Check	14° frost	10° frost	Check	14° frost	10° frost	Check	14° frost	10° frost	Check
%				%	%	%	lb.	lb.	lb.	gm.	gm.	gm.
34.0	Feed	Feed	Feed	16	35	99	43.0	43.0	48.0	14.54	14.32	15.50
38.1	Feed	Feed	6	9	16	100	47.5	49.0	51.0	16.85	16.91	18.50
40.9	Feed	Feed	4	39	47	98	54.0	53.5	56.0	21.20	21.18	22.20
45.5	Feed	Feed	4	55	79	97	57.0	57.0	58.5	23.64	24.23	25.40
46.7	Feed	Feed	4	64	85	96	59.0	58.5	60.0	26.59	27.24	26.98
50.5*	Feed	Feed	5	78	68	94	59.0	56.0	58.0	27.76	24.93	26.34
51.1*	Feed	--	5	75	--	96	59.5	--	57.5	26.88	--	29.56
53.3*	Feed	Feed	5	83	80	95	61.0	60.0	59.5	29.50	28.19	28.70
54.9	Feed	Feed	4	89	93	96	62.5	62.0	61.5	34.40	33.08	32.63
57.4*	Feed	Feed	3	75	85	98	61.0	61.0	62.5	33.08	33.90	32.39
58.1*	6	--	3	91	--	95	61.5	--	62.0	34.89	--	35.50
58.5	5	5	2	83	86	95	62.0	62.5	65.0	36.60	36.38	36.00
59.1*	6	--	4	95	--	96	62.0	--	64.0	35.73	--	35.55
65.5*	5	--	3	94	--	92	63.0	--	63.5	36.85	--	33.18
65.6	5	--	2	84	--	95	62.0	--	64.5	35.66	--	36.95
69.4	-	--	1	--	--	96	--	--	65.0	--	--	36.31

* Samples from north end of the field.

slightly in dry matter content, they were fairly uniform about 86%. No attempt was made to calculate these figures on an entirely uniform moisture basis.

The weight per bushel of unfrozen wheat rose from 43 to 65 pounds during the experiment, and changes were slight after the grain had reached a dry matter content of 58% at the time of cutting.

Exposure to frost lowered the weight per bushel of the wheat in most cases, although rather wide variations occurred, the check sample in one or two exceptional cases being as much as 2 pounds lighter than one of the frozen samples. The greatest differences occurred between the checks and the most severely frozen immature wheat, although even the mature samples which were subjected to frost appeared to be lighter than their checks. The latter fact has been noted by others (20, 29), the explanation offered being that the frost blisters cause a separation of the kernels and hence result in a lower weight per bushel. Whether or not this be the correct explanation, it is at least certain that the discrepancies in weight of frozen and unfrozen mature wheat are due to superficial rather than intrinsic differences in the kernels, as very little, if any, change in composition, which might be checked by freezing, occurs after such mature wheat is cut.

Weight per thousand kernels. The weights per 1000 kernels (Table 9) are all calculated on the basis of 13.5% moisture. The weights of the various check samples agreed very well with those of Series 1 samples, and while the variations between the weights of samples frozen at 4° and 8° F. and their checks were not at all consistent, those between the weights of immature samples frozen at 10° and 14° F. and their checks were quite significant. These variations in kernel weights resulted from the balance between translocation and respiration after the grain was cut, and the fact that the less severely frozen kernels were just as heavy as the unfrozen ones indicates that the frost was not severe enough to stop these processes. A discussion of the translocation - respiration relations will be presented later in the paper. There were minor variations between the weights of frozen and check kernels even in mature samples, but the nature of these variations leads to the belief that changes in the weight of kernels, as a direct or indirect result of frost, cease about the time the wheat reaches a dry matter content of 58%.

Total nitrogen in wheat. The total nitrogen results are expressed as percentages of the dry matter in Table 10, as pounds per acre in Table 11, and in a later section some of them will be discussed as grams per 1000 kernels.

Table 10.

Nitrogen fractions in wheat in relation to stage of maturity and exposure to frost

1	2	3	4	5	6	7	8	9	10	11	12	13
Dry matter content at time of cutting	Total nitrogen			Salt-soluble N as % of total N			Non-protein N as % of total N			N pptd. by trichlor-acetic acid as % of total N		
	8° frost	4° frost	Check	8° frost	4° frost	Check	8° frost	4° frost	Check	8° frost	4° frost	Check
%	%	%	%	%	%	%	%	%	%	%	%	%
31.2	2.94	3.16	3.04	34.7	30.0	30.9	14.2	10.8	10.5	20.5	19.2	20.4
34.9	2.94	3.12	3.00	29.3	27.2	28.7	12.3	10.3	10.3	17.0	16.9	18.4
39.4	2.94	3.00	3.11	28.2	26.0	26.0	12.3	10.3	10.0	15.9	15.7	16.0
43.9	2.68	2.74	2.74	25.4	24.1	25.5	11.6	10.2	10.2	13.8	13.9	15.3
46.8	2.78	2.78	2.72	25.9	25.5	24.3	10.4	10.1	9.2	15.5	15.4	15.1
48.3*	2.60	2.72	2.72	25.0	25.7	25.7	11.5	10.3	9.9	13.5	15.4	15.8
50.5*	2.57	2.58	2.67	24.9	23.3	24.0	11.7	10.5	9.4	13.2	12.8	14.6
51.1*	2.52	--	2.52	24.6	--	25.4	11.1	--	9.5	13.5	--	15.9
51.3*	2.55	2.63	2.54	23.5	23.6	25.2	10.6	9.9	9.4	12.9	12.7	15.8
55.8*	2.67	2.68	2.60	25.5	24.6	25.8	9.0	9.0	8.5	16.5	15.6	17.3
56.8	2.84	3.01	2.98	22.5	21.9	21.8	8.5	7.3	7.4	14.0	14.6	14.4
57.3	2.84	2.85	2.95	22.2	21.8	21.7	7.7	7.7	7.5	14.5	14.1	14.2
58.1*	2.76	--	2.76	23.9	--	23.6	9.1	--	8.0	14.8	--	15.6
59.1*	2.74	--	2.66	24.1	--	24.8	9.5	--	9.4	14.6	--	15.4
65.5*	2.78	--	2.70	23.7	--	25.9	9.4	--	8.9	14.3	--	17.0
65.6	3.06	--	3.04	22.9	--	23.4	9.2	--	8.2	13.7	--	15.2

* Samples from north end of the field.

Table 10 (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13
Dry matter content at time of cutting	Total nitrogen			Salt-soluble N as % of total N			Non-protein N as % of total N			N pptd. by trichloracetic acid as % of total N		
	14° frost	10° frost	Check	14° frost	10° frost	Check	14° frost	10° frost	Check	14° frost	10° frost	Check
%	%	%	%	%	%	%	%	%	%	%	%	%
34.0	2.70	2.78	2.98	34.8	35.3	29.5	21.5	18.7	10.4	13.3	16.6	19.1
38.1	2.72	2.70	3.00	30.9	30.4	26.7	18.4	17.0	10.3	12.5	12.6	16.4
40.9	2.66	2.66	2.86	27.4	27.8	25.1	15.0	14.3	9.8	12.4	13.5	15.3
45.5	2.63	2.65	2.76	25.9	25.8	23.9	11.8	11.3	9.1	14.1	14.5	14.8
46.7	2.72	2.71	2.81	26.5	25.1	24.9	11.0	10.7	9.2	15.5	14.4	15.7
50.5*	2.56	2.56	2.65	24.6	23.8	23.4	12.1	10.9	9.8	12.5	12.9	13.6
51.1*	2.47	--	2.52	24.3	--	25.4	11.1	--	9.5	13.2	--	15.9
53.3*	2.59	2.58	2.63	25.5	23.3	23.6	12.4	11.6	9.5	13.1	11.7	14.1
54.9	2.80	2.78	2.87	22.1	21.6	21.6	8.6	8.6	7.7	13.5	13.0	13.9
57.4*	2.63	2.65	2.69	24.3	24.1	24.5	9.9	9.3	8.2	14.4	14.8	16.3
58.1*	2.76	--	2.76	23.9	--	23.6	9.1	--	8.0	14.8	--	15.6
58.5	3.05	3.00	3.02	21.6	22.0	21.9	7.9	8.0	7.3	13.7	14.0	14.6
59.1*	2.73	--	2.66	24.2	--	24.8	9.5	--	9.4	14.7	--	15.4
65.5*	2.78	--	2.70	23.7	--	25.9	9.4	--	8.9	14.3	--	17.0
65.6	3.06	--	3.04	22.9	--	23.4	9.2	--	8.2	13.7	--	15.2
69.4	--	--	3.02	--	--	--	-	--	-	--	--	--

* Samples from north end of the field.

The percentage results for the check samples show the same general trend as those for air-dried samples in Series 1, although more of the immature samples in this series contain high percentages of nitrogen than did the Series 1 samples containing the same amount of dry matter at cutting. It was pointed out that the high values for nitrogen in the more immature wheat were the result of carbohydrate losses due to respiration. Drying was undoubtedly slower in the stook than under laboratory conditions and the probability of increased respiration will account for the higher percentage of nitrogen in the stook-dried samples of Series 3. The nitrogen content of the mature samples collected at the south end of the field is the same as for the mature samples of Series 1. This fact gives additional proof for the statement that changes in the nitrogen content of wheat cease as soon as the wheat contains about 56 to 60% dry matter.

Exposure to 4° frost did not seem to have any effect on the total nitrogen content of the wheat, but the more severely frozen immature samples were all lower in percentage nitrogen than their checks. These lower values were probably not directly the result of changes brought about by freezing, but rather indirectly due to the frost inasmuch as it caused an abrupt cessation of translocation, and reduced carbohydrate losses by

respiration. Since it is apparent that the high percentages of nitrogen in unfrozen wheat were due to respiration losses, anything which tended to reduce these losses of carbohydrates would reduce the rise in percentage nitrogen during drying. There were consistent and relatively large differences between the nitrogen content of severely frozen and check samples, the latter being higher in all those samples containing less than 58% dry matter at the time they were exposed to frost. From this stage until maturity, however, no such differences could be noted in the results, and it is clear that the factors causing these variations in the more immature wheat were not operative in the riper samples. It has already been noted that translocation and respiration effects, for all practical purposes, ceased as soon as the wheat reached the stage of development at which rapid desiccation set in. That this stage was the same as that at which frost ceased to cause variations in nitrogen content of the wheat, is additional proof that these processes were directly responsible for the variations.

The yield of wheat and nitrogen per acre for all check samples collected at the south end of the plot are presented in Table 11. Accurate yields were obtained for the first ten cuttings, but those for the last six recorded in the table had to be calculated from these ten, because the portion of the plot from which they were

Table 11.

Yield of wheat and nitrogen per acre from check samples cut at the south end of the field

1	2	3	4
Dry matter content at cutting %	Yield of wheat per acre bu.	Total nitrogen in wheat %	Yield of nitrogen per acre lb.
31.2	11.9	3.04	21.7
34.0	12.2	2.98	21.8
34.9	14.7	3.00	26.7
38.1	15.5	3.00	27.9
39.4	19.9	3.11	37.1
40.9	18.9	2.86	32.4
43.9	23.8	2.74	39.1
45.5	26.5	2.76	43.9
46.8	28.1	2.72	45.8
46.7	32.5	2.81	54.8
54.9	36.2	2.87	62.3
56.8	39.2	2.98	70.1
57.3	40.4	2.95	71.5
58.5	40.0	3.02	72.5
65.6	41.0	3.04	74.9
69.4	40.3	3.02	73.0

collected was uneven and broken. The only assumption made in the calculations is that the yield should be proportionate to the weight per 1000 kernels. The ratios between yield and weight per 1000 kernels for the eighth, ninth, and tenth cuttings were calculated and an average of these ratios used in determining the yield for the last six cuttings.

The yield per acre reached a constant value by the time the wheat contained 57% dry matter. The yield of nitrogen per acre appears to increase slightly until a later date, but analytical results from Series 1 indicate that the weight of nitrogen in the crop would not increase once the total weight had reached a maximum. It is probably safe to state that a maximum yield of grain and of nitrogen in the grain may be obtained by cutting wheat any time after it contains 57 to 58% dry matter.

Nitrogen fractions in wheat. The analytical results for the lower nitrogen fractions (Table 10) are all expressed as percentages of total nitrogen, and as the various fractions are similar in trend, although different in absolute values, they will be discussed together.

The amounts of these lower fractions in the immature check samples were lower than those in the air-dried material of Series 1. This indicates that

greater proportions of these fractions were utilized in synthesis of higher compounds during the slow drying in the stook than during the more rapid air-drying in the laboratory. The amounts of these fractions in the mature wheat were similar for both Series 1 and 3 samples. This final constant amount was also found in the frozen samples.

As was the case with total nitrogen, so with these fractions, 4° frost did not affect the percentage amounts in the wheat, but the more severe frosts did. All those samples subjected to 8°, 10° and 14° F. frost before they contained 56-58% dry matter, were higher in these fractions than their checks. The synthesis which took place after cutting must have been less in the frozen than in non-frozen wheat, and a comparison of the results for these severely frozen samples with those for the fresh kernels in Series 1 shows that although synthesis may not have been entirely stopped by the frost, it was reduced so much that it was almost negligible in comparison with that which took place in the check samples. This conclusion is further substantiated by the results for the nitrogen precipitated from the salt-solution extract by trichloracetic acid. The amounts of this fraction precipitated from the check extracts were greater than those from the frozen sample extracts. This means that more of the extractable material of the checks than

of the frozen samples was of protein nature, a condition which could exist only if synthesis were more active in the non-frozen kernels.

The differences between the results for frozen and check material disappeared in all samples which had reached 56 to 58% dry matter before being cut and subjected to frost.

Nitrogen in flour. The flours milled from Series 3 wheat samples were analysed by the same methods as the wheat, and the results are presented in Table 12.

The amounts of total nitrogen and fractions in flours from check and 4° frozen samples were essentially the same, but those in flours milled from severely frozen wheat showed differences. The total nitrogen was lower, and the salt-soluble and non-protein nitrogen were higher in the frozen material. As in the wheat, however, these differences between results for check and frozen samples disappeared in all flour milled from wheat which contained 58% dry matter at cutting.

It has already been stated that 4° frost does not seem to change the composition of the wheat, although the commercial grade under present standards is lowered. Probably, since the flour shows no composition changes, any effect the 4° frost had on the wheat was confined to the bran layers. The more severe frosts, however, not only caused changes in the bran layers, but influenced the

Table 12.

Nitrogen fractions in flour milled from wheat samples described in table 9

1	2	3	4	5	6	7	8	9	10	11	12	13
Dry matter content at time of cutting	Total nitrogen			Salt-soluble N as % of total N			Non-protein N as % of total N			N pptd. by trichlor-acetic acid as % of total N		
	8° frost	4° frost	Check	8° frost	4° frost	Check	8° frost	4° frost	Check	8° frost	4° frost	Check
%	%	%	%									
31.2	2.76	3.08	2.97	21.0	18.8	19.9	8.7	6.2	6.1	12.3	12.6	13.8
34.9	2.74	2.86	2.88	20.1	17.5	18.1	7.7	5.6	6.2	12.4	10.9	11.9
39.4	2.90	2.94	3.10	17.2	14.6	15.5	6.2	4.8	5.2	11.0	9.8	10.3
43.9	2.59	2.58	2.70	18.5	15.5	14.8	6.6	5.0	4.4	11.9	10.5	10.4
46.8	2.68	2.74	2.74	16.4	15.3	15.7	6.0	5.5	5.1	10.4	9.8	10.6
48.3*	2.46	2.60	2.60	17.1	17.3	18.1	6.9	6.5	6.5	10.2	10.8	11.6
50.5*	2.42	2.44	2.58	16.5	15.2	14.7	7.4	5.9	5.4	9.1	9.3	9.3
51.1*	2.34	--	2.48	17.9	--	16.1	7.7	-	6.0	10.2	--	10.1
51.3*	2.47	2.41	2.40	17.4	16.6	16.7	7.7	6.6	6.2	9.7	10.1	10.5
55.8*	2.70	2.63	2.68	15.6	15.6	15.2	7.4	6.1	5.2	8.2	9.5	10.0
56.8	2.68	2.82	2.93	13.4	12.8	13.3	5.2	4.3	4.8	8.2	8.5	8.5
57.3	2.63	2.67	2.67	14.4	15.7	14.2	5.0	5.2	4.5	9.4	10.5	9.7
58.1*	2.50	--	2.74	12.8	--	14.9	4.8	-	5.1	8.0	--	9.8
59.1*	2.58	--	2.50	14.5	--	15.2	6.4	-	5.6	8.1	--	9.6
65.5*	2.52	--	2.52	16.7	--	16.7	5.6	-	5.2	11.1	--	11.5
65.6	2.83	--	2.84	15.1	--	14.4	4.6	-	4.2	10.5	--	10.2

* Samples from north end of the field.

Table 12 (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13
Dry matter content at time of cutting	Total nitrogen			Salt-soluble N as % of total N			Non-protein N as % of total N			N pptd. by trichlor-acetic acid as % of total N		
	14° frost	10° frost	Check	14° frost	10° frost	Check	14° frost	10° frost	Check	14° frost	10° frost	Check
%												
34.0	2.56	2.60	2.82	27.3	26.5	18.4	16.4	13.5	6.0	10.9	13.0	12.4
38.1	2.56	2.56	2.84	25.8	22.7	17.3	14.1	12.5	6.3	11.7	10.2	11.0
40.9	2.56	2.50	2.73	19.9	19.6	15.4	10.2	8.4	5.1	9.7	11.2	10.3
45.5	2.52	2.46	2.64	17.5	16.7	15.2	7.5	6.5	5.3	10.0	10.2	9.9
46.7	2.60	2.63	2.74	15.4	14.8	17.5	6.5	6.8	5.5	8.9	8.0	12.0
50.5*	2.45	2.42	2.52	18.8	17.4	17.5	8.6	7.4	6.3	10.2	10.0	11.2
51.1*	2.40	--	2.48	18.3	--	16.1	9.2	-	6.0	9.1	--	10.1
53.3*	2.49	2.51	2.46	20.4	18.3	17.9	9.6	8.0	6.5	10.8	10.3	11.4
54.9	2.48	2.50	2.46	17.7	16.4	17.5	6.5	5.6	5.7	11.2	10.8	11.8
57.4*	2.46	2.49	2.46	14.6	12.0	13.8	6.3	5.2	4.9	8.3	6.8	8.9
58.1*	2.50	--	2.74	12.4	--	14.9	5.2	-	5.1	7.2	--	9.8
58.5	2.82	2.79	2.82	11.3	11.5	11.7	5.0	5.0	4.6	6.3	6.5	7.1
59.1*	2.58	--	2.50	15.1	--	15.2	6.2	-	5.6	8.9	--	9.6
65.5*	2.42	--	2.52	16.9	--	16.7	6.2	-	5.2	10.7	--	11.5
65.6	2.83	--	2.84	15.5	--	14.4	5.3	-	4.2	10.2	--	10.2
69.4	--	--	2.84	--	--	14.8	-	-	4.2	--	--	10.6

* Samples from north end of the field.

development of the endosperm and hence the nature of the resulting flour.

These results would explain why Shutt (32) working with lightly frozen wheats found that differences in nitrogen fractions in frozen and non-frozen wheat disappeared in the flour, while others (5, 28) whose samples of wheat were more severely frozen have found that these differences persist in the flour. The degree of frost and the stage of maturity of the wheat when frozen determine whether or not the flour milled from frozen wheat will be abnormal.

Gluten in flour. The flours milled from Series 3 samples are excellent material for a study of the washed gluten as affected by maturity and frost. This study has been started but not completed. It is proposed to carry it out on all samples in the series. The results so far obtained suggest that the following are the most important effects of maturity and frost on the gluten.

The washed gluten in immature check samples was only slightly less in quantity, and slightly less firm and tenacious in quality than that from mature non-frozen samples. That in the lightly frozen immature samples was not reduced in quantity, but instead of retaining its firm tenacious nature during washing, it became very soft and slack. The gluten in severely frozen immature samples

was reduced in quantity, and in washing became so slack that it was almost impossible to handle. The gluten in flours from frozen mature samples showed no differences from that in the check flours.

In the earlier samples, frozen and check, the dry gluten, as a percentage of the flour, was lower than the crude protein (N. x 5.7), but in the mature samples the reverse relation existed.

Further conclusions may be forthcoming when the work on the entire series is completed.

Ash. There was a gradual decrease in ash content of the kernels as they developed, the final percentage ash being only half as large as the initial percentage (Table 13). This decrease appears to have continued even after the wheat had become fully developed and mature, but why changes in ash should have occurred after other constituents had reached a final, fairly definite level, is not at all clear.

The results for frozen samples in some cases showed wide variations from those for checks, but since they bear no relation to treatment are probably the result merely of sampling errors. The trend is the same in both frozen samples and checks, and appears to depend on the progress of development toward maturity.

Table 13.

Ash in wheat in relation to stage of maturity and exposure to frost

1	2	3	4	1	2	3	4
Dry matter at time of cutting	Ash on basis of 13.5% moisture			Dry matter at time of cutting	Ash on basis of 13.5% moisture		
	8° frost	4° frost	Check		14° frost	10° frost	Check
%	%	%	%	%	%	%	%
31.2	2.56	2.18	2.29	34.0	2.08	2.06	2.10
34.9	1.94	1.95	2.11	38.1	1.64	1.90	1.94
39.4	1.82	1.88	1.77	40.9	1.54	1.76	1.54
43.9	1.58	1.44	1.50	45.5	1.54	1.54	1.50
46.8	1.43	1.43	1.51	46.7	1.47	1.49	1.41
48.3	1.45	1.52	1.53	50.5	1.38	1.42	1.42
50.5	1.45	1.40	1.38	51.1	1.44	-	1.43
51.1	1.47	-	1.43	53.3	1.40	1.41	-
51.3	1.39	1.39	1.43	54.9	1.31	1.31	1.49
55.8	1.43	1.38	1.37	57.4	1.35	1.33	1.30
56.8	1.31	1.35	1.31	58.1	1.31	-	1.26
57.3	1.33	1.30	1.30	58.5	1.35	1.32	1.38
58.1	1.26	-	1.26	59.1	1.35	-	1.37
59.1	1.29	-	1.37	65.5	1.27	-	1.28
65.5	1.26	-	1.28	65.6	1.18	-	1.20
65.6	1.21	-	1.20	69.4	-	-	1.18

Respiration and translocation. Throughout this paper numerous references have been made to the losses of carbohydrates by respiration, and also to the translocation of materials into the kernels after the wheat had been cut. It is, of course, an accepted fact that respiration occurs in all living material, and its operation was clearly demonstrated in Series 1 samples. There is no reason to suppose that there would be any differences in Series 3 samples unless the differences were caused by the frost effects. Freezing must influence the respiratory activities of the grain, as many of the results obtained cannot be accounted for by any other explanation. It is very unlikely that the frost stops respiration entirely, because every sample in the series contained some kernels capable of germination and which were therefore alive. Furthermore, the fact that the nitrogen percentage in immature frozen kernels was higher than that in comparable Series 1 material analysed fresh, indicates that respiration caused a certain loss of carbohydrates from the frozen kernels. This loss is much smaller than that from the unfrozen checks.

The results for weights of total nitrogen and dry matter per 1000 kernels of wheat subjected to 10° and 14° of frost and their checks, suggested that translocation must have taken place in the check samples after they had been cut and stooked. In the immature

wheat the weights of the nitrogen and dry matter in the checks was consistently higher than in the frozen samples. The figures for weight of nitrogen (here expressed as protein) and dry matter per 1000 kernels are gathered together in Table 14. The values for frozen wheat are the averages of the results for 10° and 14° frosted samples, and the check values are those for the checks of the same cuttings. In every case in which the wheat contained less than 50% dry matter at the time of freezing, the check sample is higher in weight of dry matter, and in every case included in the table the check was higher in nitrogen than the frozen material. This was sufficient proof that translocation had occurred in the check samples after they had been cut. The next step was to attempt to estimate, roughly, the amount of translocation.

To do this it was necessary to make two assumptions. The first, that translocation and respiration activities were stopped by freezing, has already been discussed in this section. That this assumption was not altogether true is probable, but if translocation did occur after freezing then the measured differences in weight between the frozen and check samples would be smaller than the actual translocation in the check samples, and so the error would be on the conservative side. If respiration occurred after freezing then the error would be in the opposite direction. The second assumption, that the respiration of the stooked check

Table 14.

Translocation after cutting in relation to stage of maturity

1	2	3	4	5	6	7	8	9	10	11	12
Dry matter content at cutting	Weight per 1000 kernels			Weight of protein* per 1000 kernels			Net carbohydrate translocation	Loss of+ carbohydrates by respiration	Gross carbohydrate translocation	Gross total translocation	Protein as % of total translocation
	Frozen	Check	Net gain by translocation	Frozen	Check	Net gain by translocation					
34.0	12.48	13.41	0.93	1.949	2.280	0.331	0.60	1.40	2.00	2.33	14.2
38.1	14.60	16.00	1.40	2.252	2.736	0.484	0.92	1.60	2.52	3.00	16.1
40.9	18.33	19.20	0.87	2.776	3.129	0.353	0.52	1.40	1.92	2.27	15.6
45.5	20.71	21.97	1.26	3.112	3.454	0.342	0.92	0.85	1.77	2.11	16.2
46.7	23.28	23.34	0.06	3.602	3.739	0.137	-0.08	0.80	0.72	0.86	15.9
50.5	22.78	22.78	0.00	3.329	3.443	0.114	-0.11	0.75	0.64	0.75	15.2
53.3	24.95	24.82	-0.13	3.676	3.722	0.046	-0.18	0.32	0.14	0.19	24.2

* Total nitrogen x 5.7.

+ Series 1 results.

samples during drying was the same as for the air-dried samples in Series 1, has been discussed in the section on total nitrogen, where it was pointed out that respiration in the check samples was probably greater. If this is true then the error in the calculations as to the amount of material lost by respiration is also on the conservative side, as the corrections for respiration losses are too low.

The first 7 columns of Table 14 are self-explanatory. The values in column 8 were obtained by subtracting the weight of protein translocated from the net total gain as determined from weights per 1000 kernels. The figures in column 8 are termed carbohydrate gain, but they also include other constituents such as ash. The values in column 9 were calculated from Series 1 material by interpolation, as samples cut at exactly the same moisture contents were not available. The gross carbohydrate values were obtained by adding the figures in columns 8 and 9, and the gross total translocation values are the sums of total carbohydrate and protein translocation for each cutting.

In the most immature samples, the calculated translocation was about 15% of the total weight of the kernels; that is, 15% of the kernel weight was attained after the grain was cut. Although this percentage decreased rapidly with development, there was measureable

translocation at least until the time the grain contained 50% dry matter at cutting.

The percentage protein in the translocated material was very constant during the whole course of development, and is very close to the percentage of protein (total nitrogen x 5.7) in the complete kernels. The high value for the last sample included in Table 14 was subject to large errors in calculation, on account of the very small amounts of materials translocated.

It has been indirectly inferred in previous sections of the paper that the ratio of nitrogenous to non-nitrogenous substances in the material moved into the developing endosperm was constant during the whole period of development, and that changes noted in the percentage nitrogen were caused by respiration losses. The calculations of protein and total translocation are in harmony with this belief, which was in part put forward by Brenchley and Hall in 1909 (12). Any changes which do occur in the ratio of nitrogenous to non-nitrogenous substances in the translocated material, during the course of development of the endosperm, are small.

Conclusions. 1. The commercial grade of wheat improves during the time the dry matter content is changing from 40 to 60%. More immature wheat will grade feed, and more mature will grade the same as that cut while containing 60% dry matter. Frost reduces the grade

of wheat even in those samples apparently unaffected in other ways.

2. Germination is not influenced by the maturity of the wheat at cutting, but is reduced by severe frost.

3. The weight per bushel of non-frozen wheat gradually increases until the dry matter content reaches 58%. Frozen wheat is in general lower in weight per bushel than non-frozen.

4. Wheat kernels subjected to light frost are not affected in weight, but those exposed to severe frost are lighter than non-frozen kernels.

5. The percentage total nitrogen in frozen samples is lower than in the checks but in samples frozen at 4° is not affected. The differences are due to the effects of the frost on respiration and translocation, rather than to direct changes in nitrogen as a result of freezing.

6. The yield of grain and protein practically reach a maximum by the time the kernels contain 58% dry matter.

7. The lower nitrogen fractions are present in greater quantities in severely frozen samples than in the checks, but the differences disappear in samples frozen after they contain 58% dry matter.

8. The total nitrogen and its fractions in the flours milled from severely frozen samples show the same variations as in the wheat, but the amounts in flours from

lightly frozen material are the same as in the checks.

9. Frost appears to have no effect on the ash content of the kernels, which in both frozen and unfrozen samples diminishes with progress toward maturity.

10. Respiratory activities persist to a certain extent in frozen wheat, but severe frost reduces the subsequent loss of carbohydrates to practically insignificant proportions.

11. Translocation of materials from the vegetative parts of the plant to the kernels continues while immature grain is drying in the stook. The rate becomes negligible when the kernels contain over 50% dry matter, and at any stage translocation is largely stopped by severe freezing.

12. The ratio of nitrogenous to non-nitrogenous substances in the translocated material is very nearly, if not entirely, constant during the whole period of endosperm development.

SUMMARY

The conclusions drawn from individual results are presented at the end of the discussion of each series, and this summary states only the broader, more practical aspects of the questions of development and frost effects on wheat.

The results of Series 1 and 3 indicate that there is a critical stage in the development of wheat kernels, marked by a moisture content of about 58%. This stage is of interest from both the scientific and practical points of view. Wheat cut before this stage has been reached is not fully developed, but that cut at any later period seems to have acquired all the characteristics which are found in the same wheat allowed to mature fully before cutting.

Rapid desiccation of the kernels begins at the time the critical stage is reached. Although this abrupt change in moisture content may not be entirely responsible for the cessation of other developmental factors, it is undoubtedly closely related to them.

The effects of frost on composition and quality disappear in all samples frozen after this stage of development has been reached. Grade, however, which is the criterion for the relative prices paid for wheat, is subject to change even in mature samples if these are exposed to severe frost. This is probably the most serious aspect of the problem of equitable merchandizing of frozen wheat. If it were possible to bring the grade of individual shipments into line with their actual value to the miller and baker, then the present discrepancies between grade and quality would of necessity disappear. This would mean that the miller could no longer obtain a certain amount of high quality grain at bargain prices, but

would pay according to the quality of the wheat bought. The baker would lose nothing, while the farmer, in many instances, would obtain a much fairer price for his frozen wheat than he does at present.

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